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*[Continued on next page]*

(54) Title: NOVEL HUMAN HISTONE DEACETYLASES

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   GlyIleAlaTyrAspProLeuMetLeuLysHisGlnCysValCysGly
1  ggaattgcctatgacccttgatgctgaaacaccagtgcgtttgtggc
   ccttaacggatactggggaactacgactttgtggtcacgcaaacaccg

   AsnSerThrThrHisProGluHisAlaGlyArgIleGlnSerIleTrp
49 aattccaccacccaccctgagcatgctggacgaatacagagtatctgg
   ttaaggtggtgggtgggactcgtacgacctgcttatgtctcatagacc

   SerArgLeuGlnGluThrGlyLeuLeuAsnLysCysGluArgIleGln
97 tcacgactgcaagaaactgggctgctaaataaatgtgagcgaattcaa
   agtgctgacgttctttgacccgacgatttatttacactcgcttaagtt

   GlyArgLysAlaSerLeuGluGluIleGlnLeuValHisSerGluHis
145 ggtcgaaaagccagcctggaggaaatacagcttgttcattctgaacat
    ccagcttttcggtcggacctcctttatgtcgaacaagtaagacttgta

   HisSerLeuLeuTyrGlyThrAsnProLeuAspGlyGlnLysLeuAsp
193 cactcactgttgtatggcaccaacccccctggacggacagaagctggac
    gtgagtgacaacataccgtggttgggggacctgcctgtcttcgacctg

   ProArgIleLeuLeuGlyAspAspSerGlnLysPhePheSerSerLeu
241 cccaggatactcctaggtgatgactctcaaaagtttttttcctcatta
    gggtcctatgaggatccactactgagagttttcaaaaaaaggagtaat

   ProCysGlyGlyLeuGlyValSerThr
289 ccttgtggtggacttggggtaagtaca
    ggaacaccacctgaaccccattcatgt
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(57) Abstract: The present invention relates to newly discovered human histone deacetylases (HDACs), also referred to as histone deacetylase-like polypeptides. The polynucleotide sequences and encoded polypeptides of the novel HDACs are encompassed by the invention, as well as vectors comprising these polynucleotides and host cells comprising these vectors. The invention also relates to antibodies that bind to the disclosed HDAC polypeptides, and methods employing these antibodies. Also related are methods of screening for modulators, such as inhibitors or antagonists, or agonists. The invention also relates to diagnostic and therapeutic applications which employ the disclosed HDAC polynucleotides, polypeptides, and antibodies, and HDAC modulators. Such applications can be used with diseases and disorders associated with abnormal cell growth or proliferation, cell differentiation, and cell survival, e.g., neoplastic cell growth, and especially breast and prostate cancers or tumors.

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# NOVEL HUMAN HISTONE DEACETYLASES

## RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application Serial No. 60/298,296, filed June 14, 2001, which is incorporated by reference in its entirety.

## FIELD OF THE INVENTION

The present invention relates to novel members of the histone deacetylase (HDAC) family, including BMY_HDAL1, BMY_HDAL2, BMY_HDAL3, BMY_HDACX_v1, BMY_HDACX_v2, and HDAC9c. Specifically related are nucleic acids encoding the polypeptide sequences, vectors comprising the nucleic acid sequences, and antibodies that bind to the encoded polypeptides. In addition, the invention relates to pharmaceutical compositions and diagnostic reagents comprising one or more of the disclosed HDAC components. The present invention also relates to methods of treating a disease or disorder caused by malfunction of an HDAC, e.g., due to mutation or altered gene expression. The invention further relates to methods of using a modulator of an HDAC of the present invention to treat or ameliorate a disease state. Also related are methods for devising antisense therapies and prophylactic treatments using the HDACs of the invention. In particular, the disclosed HDAC components and methods may be used to prevent, diagnose, and treat diseases and disorders associated with abnormal cell growth or proliferation, cell differentiation, or cell survival, e.g., neoplasias, cancers, and tumors, such as breast and prostate cancers or tumors, and neurodegerative diseases.

## BACKGROUND OF THE INVENTION

Chromatin is a dynamic protein-DNA complex which is modulated by post-translational modifications. These modifications, in turn, regulate cellular processes such as gene transcription and replication. Key chromatin modifications include the acetylation and deacetylation of nucelosomal histone proteins. Acetylation is catalyzed by histone acetylases (HATs), whereas deacetylation is catalyzed by deacetylases (HDACs or HDAs). HDACs catalyze the removal of acetyl groups from the N-termini of histone

core proteins to produce more negatively charged chromatin. This results in chromatin compaction, which shuts down gene transcription. In addition, inhibition of HDACs results in the accumulation of hyperacetylated histones. This, in turn, is implicated in a variety of cellular responses, including altered gene expression, cell differentiation, and cell-cycle arrest (see, generally, S.G. Gray et al., 2001, *Exp. Cell Res.* 262(2):75-83, and U.S. Patent Nos. 6,110,697 and 6,068,987 to Dulski et al.).

The HDAC gene family is composed of two distinct classes. Class I HDACs are related to the yeast transcriptional regulator, RPD3. Class II HDACs include a subgroup of proteins containing a C-terminal catalytic domain as well as a separate N-terminal domain with transcriptional repression activity. Class III HDAC proteins are related to the yeast sir2 protein and require NAD for activity. Class I HDACs are predominantly nuclear, whereas class II HDACs are transported between the cytoplasm and nucleus as part of the regulation of cellular proliferation and/or differentiation (reviewed in S. Khochbin et al., 2001, *Curr. Opin. Genet. Dev.* 11(2):162-6).

The best characterized substrates for HDACs include histone or histone-like peptide sequences containing N-terminal lysines. However, non-histone HDAC substrates have also been identified, including several transcription factors. Non-histone substrates for HDACs include p53, androgen receptor, LEF1/TCF4 (B.R. Henderson et al., 2002, *J. Biol. Chem.*, published online on May 1, 2002 as Manuscript M110602200), GATA-1, and estrogen receptor-alpha (reviewed in D.M. Vigushin et al., 2002, *Anticancer Drugs* 13(1):1-13). For these substrates, deacetylation has been shown to regulate DNA/protein interactions or protein stability. Such molecules may therefore represent therapeutic targets of HDACs. Importantly, the histone deacetylase function of HDACs represses transcription by removing the acetyl moieties from amino terminal lysines on histones, thereby resulting in a compact chromatin structure. In contrast, the non-histone deacetylase function of HDACs can either repress or activate transcription.

There has been considerable interest in modulating the activity of HDACs for the treatment of a variety of diseases, particularly cancer. Several

small molecule inhibitors of HDAC have shown anti-proliferative activities on a number of tumor cell lines and potent anti-tumor activity in pre-clinical tumor xenograft models, most recently, CBHA (D.C. Coffey et al., 2001, *Cancer Res.* 61(9):3591-4), pyroxamide, (L.M. Butler et al, 2001, *Clin. Cancer Res.* 7(4):962-70), and CHAP31 (Y. Komatsu et al., 2001, *Cancer Res.* 61(11):4459-66). Several inhibitors are presently being evaluated as single agents and in combination regimens with cytotoxic agents for the treatment of advanced malignancies (reviewed in P.A. Marks et al., *Curr. Opin. Oncol.* 2001 Nov;13(6):477-83). Thus, HDAC inhibitors are being developed as anti-tumor agents, as well as agents useful for gene therapy (McInerney et al., 2000, *Gene Ther.* 7(8):653-663).

Small molecule inhibitors of HDAC activity that have undergone extensive analysis include trichostatin A (TSA), trapoxin, SAHA (V.M. Richon et al., 2001, *Blood Cells Mol. Dis.* 27(1):260-4), CHAPs (Y. Komatsu et al., 2001, *Cancer Res.* 61(11):4459-66), MS-27-275 (reviewed in M. Yoshida et al., 2001, *Cancer Chemother. Pharmacol.* 48 Suppl. 1:S20-6), depsipeptide (FR901228; FK228; see, e.g., V. Sandor et al., 2002, *Clin. Cancer Res.* 8(3):718-28), and CI-994 (see, e.g., P.M. LoRusso et al., 1996, *New Drugs* 14(4):349-56; S. Prakash et al., 2001, *Invest. New Drugs* 19(1):1-11). Trichostatin A and trapoxin have been reported to be reversible and irreversible inhibitors, respectively, of mammalian histone deacetylase (Yoshida et al, 1995, *Bioassays*, 17(5):423-430). Trichostatin A has also been reported to inhibit partially purified yeast histone deacetylase (Sanchez del Pino et al., 1994, *Biochem. J.*, 303:723-729). Moreover, trichostatin A is an antifungal antibiotic and has been shown to have anti-trichomonal activity and cell differentiating activity in murine erythroleukemia cells, as well as the ability to induce phenotypic reversion in ras-transformed fibroblast cells (see e.g. U.S. Pat. No. 4,218,478; and Yoshida et al., 1995, *Bioassays*, 17(5):423-430, and references cited therein). Trapoxin A, a cyclic tetrapeptide, induces morphological reversion of v-sis-transformed NIH/3T3 cells (Yoshida and Sugita, 1992, *Jap. J. Cancer Res.*, 83(4):324-328).

The therapeutic effects of HDAC inhibition are believed to occur through the induction of differentiation and/or apoptosis through the up-regulation of genes such as the cyclin dependent kinase inhibitors, p21 and p27 (see, e.g., W. Wharton et al., 2000, *J. Biol. Chem.* 275(43):33981-7; L. Huang et al., 2000, *Mol. Med.* 6(10):849-66). Although known HDAC inhibitors are efficacious as anti-tumor agents, they are also associated with toxicity (see, e.g., V. Sandor et al., 2002, *Clin. Cancer Res.* 8(3):718-28). Such toxicity is believed to be caused by a non-selective mechanism of targeting multiple HDACs. Despite the potent anti-tumor activity of HDAC inhibitors, it is still unclear which HDACs are necessary to produce an anti-proliferative response. Furthermore, little progress has been made in comparing the HDAC gene expression profiles in tumor versus normal cells. Differential HDAC expression may underlie the tumor-selective responses of HDAC inhibition. In addition, a cellular growth advantage may be conferred by the expression of particular HDACs. Therefore, there is a need for further insight into the consequences of selective HDAC inhibition, or activation.

## SUMMARY OF THE INVENTION

The present invention provides novel histone deacetylase (HDAC) nucleic acid sequences and their encoded polypeptide products, also called histone deacetylase like (HDAL) sequences and products herein, as well as methods and reagents for modulating HDACs.

It is an aspect of this invention to provide new HDAC nucleic acid or protein sequences, or cell lines overexpressing HDAC nucleic acid and/or encoded protein, for use in assays to identify small molecules which modulate HDAC activity, preferably antagonize HDAC activity.

It is another aspect of the present invention to employ HDAC protein structural data for the *in silico* identification of small molecules which modulate HDAC activity. This structural data could be generated by experimental techniques (for example, X-Ray crystallography or NMR spectroscopy) or by computational modeling based on available histone deacetylase structures (for example, M.S. Finnin et al., 1999, *Nature*, 401(6749):188-193).

Another aspect of the present invention provides modulators of HDAC activity, e.g., antagonists or inhibitors, and their use to treat neoplastic cells, e.g., cancer cells and tumor cells. In one aspect of the invention, breast or prostate cancers or tumors are treated using the HDAC modulators. The modulators of the invention can be employed alone or in combination with standard anti-cancer regimens for neoplastic cell, e.g., tumor and cancer, treatments.

In addition, the present invention provides diagnostic reagents (i.e., biomarkers) for the detection of cancers, tumors, or neoplastic growth. In one embodiment, HDAC (e.g., HDAC9c) nucleic acids or anti-HDAC antibodies are used to detect the presence of specific cancers or tumors, such as breast or prostate cancers or tumors.

It is yet another aspect of the present invention to employ HDAC inhibitors in the regulation of the differentiation state of normal cells such as hematopoietic stem cells. According to this invention, a method is provided for the use of modulators of HDAC in *ex vivo* therapies, particularly as a means to modulate the expression of gene therapeutic vectors.

Yet another aspect of this invention is to provide antisense nucleic acids and oligonucleotides for use in the regulation of HDAC and HDAL gene transcription or translation.

An additional aspect of this invention pertains to the use of HDAC nucleic acid sequences and antibodies directed against the produced protein for prognosis or susceptibility for certain disorders (e.g., breast or prostate cancer).

Further aspects, features and advantages of the present invention will be better appreciated upon a reading of the detailed description of the invention when considered in connection with the accompanying figures/drawings.

## BRIEF DESCRIPTION OF THE FIGURES

The file of this patent contains at least one figure executed in color. Copies of this patent with color figure(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

**FIG. 1** shows the novel BMY_HDAL1 partial nucleic acid (cDNA) sequence (SEQ ID NO:1) and the encoded amino acid sequence (SEQ ID NO:2) of the BMY_HDAL1 polypeptide product. The top line in each group of Fig. 1 presents the BMY_HDAL1 protein sequence (SEQ ID NO:2) in 3-letter IUPAC form; the middle line presents the nucleotide sequence of the BMY_HDAL1 coding strand (i.e., SEQ ID NO:1); and the bottom line presents the nucleotide sequence of the reverse strand (SEQ ID NO:3).

**FIGS. 2A and 2B** show the amino acid sequences of the novel histone deacetylase-like proteins BMY_HDAL1 (SEQ ID NO:2), BMY_HDAL2 (SEQ ID NO:4) and BMY_HDAL3 (SEQ ID NO:5) aligned with the following known histone deacetylase proteins: *S. cerevisiae* HDA1 (SC_HDA1), (SEQ ID NO:6); human HDAC4 (HDA4), (SEQ ID NO:7); human HDAC5 (HDA5), (SEQ ID NO:8); human HDAC7 (HDA7), (SEQ ID NO:9) and to a histone deacetylase-like protein ACUC from *Aquifex aeolicus* (AQUIFEX_HDAL), (SEQ ID NO:10), (M.S. Finnin et al., 1999, *Nature*, 401(6749):188-193). Residues identical among all proteins are in shown in black text on a gray background. The sequences were aligned using the ClustalW algorithm as implemented in the VectorNTI sequence analysis package (1998, 5.5 Ed., Informax, Inc.) with a gap opening penalty of 10, a gap extension penalty of 0.1 and no end gap penalties.

**FIGS. 3A and 3B** show a GenewiseDB comparison of BMY_HDAL1 amino acid sequence (SEQ ID NO:2) and human HDAC5 (HDA5) amino acid sequence (SEQ ID NO:8). Genewise results from HDA5_HUMAN_run2 applied to AC002088 nucleic acid (coding) sequence. (SEQ ID NO:11).

**FIG. 4** presents the results of sequence motif analysis of motifs within the BMY_HDAL1 amino acid sequence.

**FIG. 5** shows the novel BMY_HDAL2 partial nucleic acid (cDNA) sequence (SEQ ID NO:12) and the encoded amino acid sequence (SEQ ID NO:4) of the BMY_HDAL2 polypeptide product. The top line in each group of Fig. 5 presents the BMY_HDAL2 protein sequence (SEQ ID NO:4) in 3-letter IUPAC form; the middle line presents the nucleotide sequence of the

BMY_HDAL2 coding strand (i.e., SEQ ID NO:12); and the bottom line presents the nucleotide sequence of the reverse strand (SEQ ID NO:13).

**FIG. 6** presents a GenewiseDB comparison of the BMY_HDAL2 amino acid sequence (SEQ ID NO:4) and human HDAC5 (HDA5) amino acid sequence (SEQ ID NO:8). Genewise results from HDA5_HUMAN_run3 applied to AC002410 nucleic acid sequence (SEQ ID NO:14).

**FIG. 7** shows PROSITE motifs identified in the predicted amino acid sequence of the novel BMY_HDAL2 (SEQ ID NO:4). MOTIFS are from: bmy_hdal2.aa.fasta.

**FIGS. 8A and 8B** show the sequences of the N- and C-terminal sequences of BMY_HDAL3 as determined from BAC AC004994 and BAC AC004744. **FIG. 8A** presents the most N-terminal region of the BMY_HDAL3 amino acid sequence (SEQ ID NO:15) presented herein as encoded by the human genomic BAC AC004994 polynucleotide sequence (SEQ ID NO:17). **FIG. 8B** presents an additional C-terminal portion of the BMY_HDAL3 amino acid sequence (SEQ ID NO:16) as encoded by human genomic BAC AC004744 polynucleotide sequence (SEQ ID NO:18).

**FIG. 9** shows partial transcripts identified from the AC004994 polynucleotide sequence (SEQ ID NO:17) and from the AC004744 polynucleotide sequence (SEQ ID NO:18) assembled into a single contig, which was designated BMY_HDAL3 (SEQ ID NO:19) using the VectorNTI ContigExpress program (Informax, Inc.).

**FIG. 10** presents the BMY_HDAL3 partial nucleic acid sequence (SEQ ID NO:19) and the encoded amino acid sequence (SEQ ID NO:5) based on the assembled BMY_HDAL3 sequence described in FIG. 9. The top line in each group of FIG. 10 presents the BMY_HDAL3 protein sequence (SEQ ID NO:5) in 3-letter IUPAC form; the middle line presents the nucleotide sequence of the BMY_HDAL3 coding strand (i.e., SEQ ID NO:19); and the bottom line presents the nucleotide sequence of the reverse strand (SEQ ID NO:20).

**FIG. 11** presents the results of the GCG Motifs program used to analyze the BMY_HDAL3 partial predicted amino acid sequence for motifs in

the PROSITE collection (K. Hofmann et al., 1999, *Nucleic Acids Res.*, 27(1):215-219) with no allowed mismatches.

**FIG. 12** shows a multiple sequence alignment of the novel human HDAC, BMY_HDAL3, amino acid sequence (SEQ ID NO:5) with the amino acid sequence of AAC78618 (SEQ ID NO:21) and with the amino acid sequence of AAD15364 (SEQ ID NO:22). AAC78618 is a histone deacetylase-like protein predicted by genefinding and conceptual translation of AC004994 and which was entered in Genbank. AAD15364 is a similar predicted protein derived from AC004744 and entered in Genbank. AAC78618, AAD15364 and BMY_HDAL3 were aligned using the ClustalW algorithm as implemented in the VectorNTI sequence analysis package (1998, 5.5 Ed., Informax, Inc.) with a gap opening penalty of 10, a gap extension penalty of 0.1 and no end gap penalties. Residues identical among all proteins are shown in white text on a black background; conserved residues are shown in black text on a gray background.

**FIG. 13** shows a BLASTN alignment of the AA287983 polynucleotide sequence (SEQ ID NO:23) and BMY_HDAL3 polynucleotide sequence from SEQ ID NO:19. Genbank accession AA287983 is a human EST sequence (GI # 1933807; Incyte template 1080282.1) which was identified by BLASTN searches against the Incyte LifeSeq database using the NCBI Blast algorithm (S.F. Altschul et al., 1997, *Nucl. Acids Res.*, 25(17):3389-3402) with default parameters. The AA287983 human EST was isolated from a germinal B-cell library. No additional ESTs are included in the Incyte template derived from this cluster (Incyte gene ID 180282).

**FIGS. 14A-14H** present other histone deacetylase sequences, as shown in FIGS. 2A and 2B. **FIG. 14A**: *Aquifex* ACUC protein amino acid sequence (SEQ ID NO:10); **FIG. 14B**: *Saccharomyces cerevisiae* histone deacetylase 1 amino acid sequence (SEQ ID NO:6); **FIG. 14C**: *Homo sapiens* histone deacetylase 4 amino acid sequence (SEQ ID NO:7); **FIG. 14D**: *Homo sapiens* histone deacetylase 5 amino acid sequence (SEQ ID NO:8); **FIG. 14E**: *Homo sapiens* histone deacetylase 7 amino acid sequence (SEQ ID NO:9); **FIG. 14F**: Human EST AA287983 nucleic acid sequence

(SEQ ID NO:23); **FIG. 14G**: Human predicted protein AAD15364 amino acid sequence(SEQ ID NO:22); and **FIG. 14H**: Human predicted protein AAC78618 amino acid sequence (SEQ ID NO:21).

**FIGS. 15A-15C** depict the nucleotide and amino acid sequence information for HDAC9c. The polypeptide sequence (SEQ ID NO:87) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:88) of the coding strand is also shown. **FIGS. 15D-15F** depict an amino acid sequence alignment of HDAC9c. The predicted amino acid sequence of HDAC9c (SEQ ID NO:87) was aligned to previously identified HDACs, including HDAC9 (AY032737; SEQ ID NO:89), HDAC9a (AY032738; SEQ ID NO:90), and HDAC4 (ALF132608; SEQ ID NO:91), using ClustalW (D.G. Higgins et al., 1996, *Methods Enzymol.* 266:383-402). Identical amino acids are shown in white text on a black background; conserved amino acids are shown in black text on a gray background.

**FIGS. 16A-16C** depict expression levels of HDAC9 in human cancer cell lines and normal adult tissue. **FIG 16A**: Northern blot analysis of HDAC9 expression in normal adult tissue. **FIG 16B**: Quantitative PCR mRNA analysis of HDAC9 expression in human tumor cell lines. **FIG 16C**: Nuclease protection assay analysis of HDAC9 expression in human tumor cell lines. **FIG. 16D** shows the nucleotide sequence of HDAC9c used to derive the probes used for Northern blotting and nuclease protection analysis (SEQ ID NO:92). The probes were derived from the HDAC9c nucleotide sequence, and were predicted to hybridize to HDAC9c and HDAC9 (AYO32737), but not HDAC9a (AYO32738).

**FIGS. 17A-17C** illustrate the increase of HDAC9 gene expression in human cancer tissues. **FIGS. 17A-17B**: Summary of HDAC9 expression in selected tissues, as assayed by *in situ* hybridization. **FIG. 17C**: Photomicrographs of representative cells showing HDAC9 or actin staining.

**FIG. 18** shows HDAC9c-mediated induction of morphological transformation of NIH/3T3 cells. The panels show photomicrographs of soft agar growth of vector (upper panel), FGF8 (middle panel) and HDAC9c (lower panel) transfected NIH/3T3 cells. Cells are shown at 10 X magnification.

**FIG. 19** shows HDAC9c induction of actin stress fiber formation in NIH/3T3 cells. Stable NIH/3T3 cells expressing the indicated constructs were stained with phalloidin-TRITC and visualized by fluorescent microscopy.

**FIGS. 20A-20C** depict the nucleotide and amino acid sequence information for BMY_HDACX variant 1, also called BMY_HDACX_v1 and HDACX_v1. BMY_HDACX_v1 represents a partial cDNA sequence obtained from cells expressing a transcript variant of human HDAC9. The polypeptide sequence (SEQ ID NO:93) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:94) of the coding strand is also shown.

**FIGS. 21A-21B** depict the nucleotide and amino acid sequence information for BMY_HDACX variant 2, also called BMY_HDACX_v2 and HDACX_v2. BMY_HDACX_v2 represents a full-length sequence of a novel transcript variant (i.e., splice product) of HDAC9. The polypeptide sequence (SEQ ID NO:95) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:96) of the coding strand is also shown.

**FIGS. 22A-22I** depict the nucleotide and amino acid sequence information for the previously identified HDAC9 transcript variants. **FIGS. 22A-22C**: HDAC9 variant 1 (HDAC9v1; NCBI Ref. Seq. NM_058176). The polypeptide sequence (SEQ ID NO:89) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:97) of the coding strand is also shown. **FIGS. 22D-22F**: HDAC9 variant 2 (HDAC9v2; NCBI Ref. Seq. NM_058177). The polypeptide sequence (SEQ ID NO:90) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:98) of the coding strand is also shown. **FIGS. 22G-22I**: HDAC9 variant 3 (HDAC9v3; NCBI Ref. Seq. NM_014707). The polypeptide sequence (SEQ ID NO:99) is shown using the standard 3-letter abbreviation for amino acids. The DNA sequence (SEQ ID NO:100) of the coding strand is also shown.

**FIGS. 23A-23K** depict a multiple sequence alignment of nucleotide sequences representing known and novel HDAC9 splice products. The

cDNAs for BMY_HDACX_v1 (SEQ ID NO:94) and BMY_HDACX_v2 (SEQ ID NO:96) nucleotide sequences were aligned to the three reported splice products of the HDAC9 gene, including HDAC9v1 (NCBI Ref. Seq. NM_058176; SEQ ID NO:97), HDAC9v2 (NCBI Ref .Seq. NM_058177; SEQ ID NO:98), and HDAC9v3 (NCBI Ref. Seq. NM_014707; SEQ ID NO:100) using the sequence alignment program ClustalW (D.G. Higgins et al., 1996, *Methods Enzymol.* 266:383-402). The consensus sequence is shown on the bottom line (SEQ ID NO:106). Identical nucleotides are shown in white text on a black background. Selected splice junctions are indicated below the alignment; these junctions were identified by comparison of the cDNA sequences to the assembled genomic contig NT_00798.1 using the Sim4 algorithm (L. Florea et al., 1998, *Genome Res.* 8:967-74). It is noted that the HDAC9 (AY032737) nucleotide and amino acid sequences are identical to the HDAC9v1 (NM_058176) nucleotide and amino acid sequences. Similarly, the HDAC9a (AY032738) nucleotide and amino acid sequences are identical to the HDAC9v2 (NM_058177) nucleotide and amino acid sequences.

**FIGS. 24A-24D** depict a multiple sequence alignment of amino acid sequences representing known and novel HDAC polypeptides. The amino acid sequences encoded by transcript variants BMY_HDACX_v1 (SEQ ID NO:93) and BMY_HDACX_v2 (SEQ ID NO:95) were aligned to amino acid sequences encoded by known splice variants of human histone deacetylase 9 including HDAC9v1 (NCBI Ref. Seq. NM_058176; SEQ ID NO:89), HDAC9v2 (NCBI Ref .Seq. NM_058177; SEQ ID NO:90), and HDAC9v3 (NCBI Ref. Seq. NM_014707; SEQ ID NO:99), and to human histone deacetylases 4 and 5 (HDA5, SEQ ID NO:8; HDA4, SEQ ID NO:7) using the multiple sequence alignment program ClustalW (D.G. Higgins et al., 1996, *Methods Enzymol.* 266:383-402). The consensus sequence is shown on the bottom line (SEQ ID NO:107). Residues conserved among all polypeptides are shown in white text on a black background; residues conserved in a majority of polypeptides are shown in black text on a gray background.

**FIGS. 25A-25C** depict a multiple sequence alignment of amino acid sequences showing novel HDAC polypeptides. The amino acid sequences of

BMY_HDAL1 (SEQ ID NO:2), BMY_HDAL2 (SEQ ID NO:4), BMY_HDAL3 (SEQ ID NO:5), HDAC9c (SEQ ID NO:87), HDACX_v1 (SEQ ID NO:93), and HDACX_v2 (SEQ ID NO:95) were aligned using the T-Coffee program (C. Notredame et al., 2000, *J. Mol. Biol.* 302:205-217; C. Notredame et al., 1998, *Bioinformatics* 14:407-422). Identical residues are shown in black text on a gray background.

## DESCRIPTION OF THE INVENTION

The present invention discloses several novel HDAC nucleotide sequences and encoded products. New members of the histone deacetylase protein family have been identified as having identity to known HDACs. Three new HDACs are referred to as BMY_HDAL1, BMY_HDAL2, and BMY_HDAL3 herein, wherein HDAL signifies histone deacetylase like proteins in current nomenclature. These proteins are most similar to the known human histone deacetylase, HDAC9. Novel HDAC9 splice variants, termed HDACX_v1 and HDACX_v2, have also been identified. In addition, HDAC9c, an HDAC9-related family member, has been newly identified and cloned. The nucleic acid sequences encoding the novel HDAC polypeptides are provided together with the description of the means employed to obtain these novel molecules. Such HDAC products can serve as protein deacetylases, which are useful for disease treatment and/or diagnosis of diseases and disorders associated with cell growth or proliferation, cell differentiation, and cell survival, e.g., neoplastic cell growth, cancers, and tumors.

As shown herein, HDAC9 expression is elevated in tumor cell lines, as determined by quantitative PCR analysis. Elevated expression of HDAC9 was also observed in clinical specimens of human tumor tissue compared to normal tissue, using *in situ* hybridization (ISH) and an HDAC9-specific riboprobe. Further, cell biological assessment of HDAC9c revealed that overexpression of HDAC9c confers a growth advantage to normal fibroblasts. These results indicate that HDAC9c can be used as a diagnostic marker for tumor progression and that selective HDAC9c inhibitors can be used to target specific cancer or tumor types, such as breast and prostate cancers or tumors.

Definitions

The following definitions are provided to more fully describe the present invention in its various aspects. The definitions are intended to be useful for guidance and elucidation, and are not intended to limit the disclosed invention and its embodiments.

HDAC polypeptides (or proteins) refer to the amino acid sequence of isolated, and preferably substantially purified, human histone deacetylase proteins isolated as described herein. HDACs may also be obtained from any species, preferably mammalian, including mouse, rat, non-human primates, and more preferably, human; and from a variety of sources, including natural, synthetic, semi-synthetic, or recombinant. The probes and oligos described may be used in obtaining HDACs from mammals other than humans. The present invention more particularly provides six new human HDAC family members, namely, BMY_HDAL1, BMY_HDAL2, BMY_HDAL3, HDACX_v1, HDACX_v2, and HDAC9c, their polynucleotide sequences (e.g., SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, SEQ ID NO:96, and sequences complementary thereto), and encoded products (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95).

An agonist (e.g., activator) refers to a molecule which, when bound to, or interactive with, an HDAC polypeptide, or a functional fragment thereof, increases or prolongs the duration of the effect of the HDAC polypeptide. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules that bind to and modulate the effect of an HDAC polypeptide. An antagonist (e.g., inhibitor, blocker) refers to a molecule which, when bound to, or interactive with, an HDAC polypeptide, or a functional fragment thereof, decreases or eliminates the amount or duration of the biological or immunological activity of the HDAC polypeptide. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules that decrease, reduce or eliminate the effect and/or function of an HDAC polypeptide.

"Nucleic acid sequence", as used herein, refers to an oligonucleotide, nucleotide, or polynucleotide (e.g., DNA, cDNA, RNA), and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense (coding) or antisense (non-coding) strand. By way of nonlimiting example, fragments include nucleic acid sequences that can be about 10 to 60 contiguous nucleotides in

length, preferably, at least 15-60 contiguous nucleotides in length, and also preferably include fragments that are at least 70-100 contiguous nucleotides, or which are at least 1000 contiguous nucleotides or greater in length. Nucleic acids for use as probes or primers may differ in length as described herein.

In specific embodiments, HDAC polynucleotides of the present invention can comprise at least 15, 20, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1195, 1200, 1500, 2000, 2160, 2250, 2500, 2755, or 2900 contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, SEQ ID NO:96, or a sequence complementary thereto. Additionally, a polynucleotide of the invention can comprise a specific region of a HDAC nucleotide sequence, e.g., a region encoding the C-terminal sequence of the HDAC polypeptide. Such polynucleotides can comprise, for example, nucleotides 3024-4467 of HDAC9c (SEQ ID NO:88), nucleotides 2156-3650 of HDACX_v1 (SEQ ID NO:94), nucleotides 1174-3391 of HDACX_v2 (SEQ ID NO:96), or portions or fragments thereof.

As specific examples, polynucleotides of the invention may comprise at least 183 contiguous nucleotides of SEQ ID NO:88; or at least 17 contiguous nucleotides of SEQ ID NO:96. As additional examples, the polynucleotides of the invention may comprise nucleotides 1 to 3207 of SEQ ID NO:88; nucleotides 1 to 2340 of SEQ ID NO:94; or nucleotides 307 to 1791 of SEQ ID NO:96. Further, the polynucleotides of the invention may comprise nucleotides 4 to 3207 of SEQ ID NO:88, wherein said nucleotides encode amino acids 2 to 1069 of SEQ ID NO:87 lacking the start methionine; or nucleotides 310 to 1791 of SEQ ID NO:96, wherein said nucleotides encode amino acids 2 to 495 of SEQ ID NO:95 lacking the start methionine. In addition, polynucleotides of the invention may comprise nucleotides 3024-3207 of SEQ ID NO:88; or nucleotides 1174-1791 of SEQ ID NO:96.

"Amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules. Amino acid sequence

fragments are typically from about 4 or 5 to about 35, preferably from about 5 to about 15 or 25 amino acids in length and, optimally, retain the biological activity or function of an HDAC polypeptide. However, it will be understood that larger amino acid fragments can be used, depending on the purpose therefor, e.g., fragments of from about 15 to about 50 or 60 amino acids, or greater.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule. In addition, the terms HDAC polypeptide and HDAC protein are frequently used interchangeably herein to refer to the encoded product of an HDAC nucleic acid sequence of the present invention.

A variant of an HDAC polypeptide can refer to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing functional biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

An allele or allelic sequence is an alternative form of an HDAC nucleic acid sequence. Alleles may result from at least one mutation in the nucleic acid sequence and may yield altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene, whether natural or recombinant, may have none, one, or many allelic forms. Common mutational changes that give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of

changes may occur alone, or in combination with the others, one or more times in a given sequence.

Altered nucleic acid sequences encoding an HDAC polypeptide include nucleic acid sequences containing deletions, insertions and/or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent HDAC polypeptide. Altered nucleic acid sequences may further include polymorphisms of the polynucleotide encoding an HDAC polypeptide; such polymorphisms may or may not be readily detectable using a particular oligonucleotide probe. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues, which produce a silent change and result in a functionally equivalent HDAC protein of the present invention. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological activity or function of the HDAC protein is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide ("oligo") linked to a peptide backbone of amino acid residues, which terminates in lysine. PNA typically comprise oligos of at least 5 nucleotides linked to amino acid residues. These small molecules stop transcript elongation by binding to their complementary strand of nucleic acid (P.E. Nielsen et al., 1993, *Anticancer Drug Des.*, 8:53-63). PNA may be pegylated to extend their lifespan in the cell where they preferentially bind to complementary single stranded DNA and RNA.

Oligonucleotides or oligomers refer to a nucleic acid sequence, preferably comprising contiguous nucleotides, typically of at least about 6 nucleotides to about 60 nucleotides, preferably at least about 8 to 10 nucleotides in length, more preferably at least about 12 nucleotides in length,

e.g., about 15 to 35 nucleotides, or about 15 to 25 nucleotides, or about 20 to 35 nucleotides, which can be typically used, for example, as probes or primers, in PCR amplification assays, hybridization assays, or in microarrays. It will be understood that the term oligonucleotide is substantially equivalent to the terms primer, probe, or amplimer, as commonly defined in the art. It will also be appreciated by those skilled in the pertinent art that a longer oligonucleotide probe, or mixtures of probes, e.g., degenerate probes, can be used to detect longer, or more complex, nucleic acid sequences, for example, genomic DNA. In such cases, the probe may comprise at least 20-200 nucleotides, preferably, at least 30-100 nucleotides, more preferably, 50-100 nucleotides.

Amplification refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies, which are well known and practiced in the art (See, D.W. Dieffenbach and G.S. Dveksler, 1995, *PCR Primer, a Laboratory Manual*, Cold Spring Harbor Press, Plainview, NY).

Microarray is an array of distinct polynucleotides or oligonucleotides synthesized on a substrate, such as paper, nylon, or other type of membrane; filter; chip; glass slide; or any other type of suitable solid support.

The term antisense refers to nucleotide sequences, and compositions containing nucleic acid sequences, which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense (i.e., complementary) nucleic acid molecules include PNA and may be produced by any method, including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes that block either transcription or translation. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term consensus refers to the sequence that reflects the most common choice of base or amino acid at each position among a series of

related DNA, RNA, or protein sequences. Areas of particularly good agreement often represent conserved functional domains.

A deletion refers to a change in either nucleotide or amino acid sequence and results in the absence of one or more nucleotides or amino acid residues. By contrast, an insertion (also termed "addition") refers to a change in a nucleotide or amino acid sequence that results in the addition of one or more nucleotides or amino acid residues, as compared with the naturally occurring molecule. A substitution refers to the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids.

A derivative nucleic acid molecule refers to the chemical modification of a nucleic acid encoding, or complementary to, an encoded HDAC polypeptide. Such modifications include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative encodes a polypeptide that retains the essential biological and/or functional characteristics of the natural molecule. A derivative polypeptide is one that is modified by glycosylation, pegylation, or any similar process that retains the biological and/or functional or immunological activity of the polypeptide from which it is derived.

The term "biologically active", i.e., functional, refers to a protein or polypeptide or peptide fragment thereof having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HDAC, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells, for example, to generate antibodies, and to bind with specific antibodies.

An HDAC-related protein refers to the HDAC and HADL proteins or polypeptides described herein, as well as other human homologs of these HDAC or HDAL sequences, in addition to orthologs and paralogs (homologs) of the HDAC or HADL sequences in other species, ranging from yeast to other mammals, e.g., homologous histone deacetylase. The term ortholog refers to genes or proteins that are homologs via speciation, e.g., closely related and assumed to have common descent based on structural and

functional considerations. Orthologous proteins function as recognizably the same activity in different species. The term paralog refers to genes or proteins that are homologs via gene duplication, e.g., duplicated variants of a gene within a genome. (See, W.M. Fritch, 1970, *Syst. Zool.*, 19:99-113.

It will be appreciated that, under certain circumstances, it may be advantageous to provide homologs of one of the novel HDAC polypeptides which function in a limited capacity as one of either an HDAC agonist (i.e., mimetic), or an HDAC antagonist, in order to promote or inhibit only a subset of the biological activities of the naturally-occurring form of the protein. Thus, specific biological effects can be elicited by treatment with a homolog of limited function, and with fewer side effects, relative to treatment with agonists or antagonists which are directed to all of the biological activities of naturally-occurring forms of HDAC proteins.

Homologs (i.e., isoforms or variants) of the novel HDAC polypeptides can be generated by mutagenesis, such as by discrete point mutation(s), or by truncation. For example, mutation can yield homologs that retain substantially the same, or merely a subset of, the biological activity of the HDAC polypeptide from which it was derived. Alternatively, antagonistic forms of the protein can be generated which are able to inhibit the function of the naturally-occurring form of the protein, such as by competitively binding to an HDAC substrate, or HDAC-associated protein. Non-limiting examples of such situations include competing with wild-type HDAC in the binding of p53 or a histone. Also, agonistic forms of the protein can be generated which are constitutively active, or have an altered $K_{cat}$ or $K_m$ for deacylation reactions. Thus, the HDAC protein and homologs thereof may be either positive or negative regulators of transcription and/or replication.

The term hybridization refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases. The hydrogen bonds may be further stabilized by base stacking

interactions. The two complementary nucleic acid sequences hydrogen bond in an anti-parallel configuration. A hybridization complex may be formed in solution (e.g., $C_0t$ or $R_0t$ analysis), or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins, or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been affixed).

The terms stringency or stringent conditions refer to the conditions for hybridization as defined by nucleic acid composition, salt and temperature. These conditions are well known in the art and may be altered to identify and/or detect identical or related polynucleotide sequences in a sample. A variety of equivalent conditions comprising either low, moderate, or high stringency depend on factors such as the length and nature of the sequence (DNA, RNA, base composition), reaction milieu (in solution or immobilized on a solid substrate), nature of the target nucleic acid (DNA, RNA, base composition), concentration of salts and the presence or absence of other reaction components (e.g., formamide, dextran sulfate and/or polyethylene glycol) and reaction temperature (within a range of from about 5°C below the melting temperature of the probe to about 20°C to 25°C below the melting temperature). One or more factors may be varied to generate conditions, either low or high stringency, that are different from but equivalent to the aforementioned conditions.

As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences. As will be further appreciated by the skilled practitioner, Tm can be approximated by the formulas as known in the art, depending on a number of parameters, such as the length of the hybrid or probe in number of nucleotides, or hybridization buffer ingredients and conditions (See, for example, T. Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982 and J. Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; *Current Protocols in Molecular Biology*, Eds. F.M. Ausubel et al., Vol. 1, "Preparation and Analysis

of DNA", John Wiley and Sons, Inc., 1994-1995, Suppls. 26, 29, 35 and 42; pp. 2.10.7- 2.10.16; G.M. Wahl and S. L. Berger (1987; *Methods Enzymol.* 152:399-407); and A.R. Kimmel, 1987; *Methods of Enzymol.*, 152:507-511). As a general guide, Tm decreases approximately 1ºC –1.5ºC with every 1% decrease in sequence homology. Also, in general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is initially performed under conditions of low stringency, followed by washes of varying, but higher stringency. Reference to hybridization stringency, e.g., high, moderate, or low stringency, typically relates to such washing conditions.

Thus, by way of nonlimiting example, high stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.018M NaCl at about 65ºC (i.e., if a hybrid is not stable in 0.018M NaCl at about 65ºC, it will not be stable under high stringency conditions). High stringency conditions can be provided, for instance, by hybridization in 50% formamide, 5 X Denhart's solution, 5 X SSPE (saline sodium phosphate EDTA) (1 X SSPE buffer comprises 0.15 M NaCl, 10 mM $Na_2HPO_4$, 1 mM EDTA), (or 1 X SSC buffer containing 150 mM NaCl, 15 mM $Na_3$ citrate • 2 $H_2O$, pH 7.0), 0.2% SDS at about 42ºC, followed by washing in 1 X SSPE (or saline sodium citrate, SSC) and 0.1% SDS at a temperature of at least about 42°C, preferably about 55°C, more preferably about 65°C.

Moderate stringency refers, by way of nonlimiting example, to conditions that permit hybridization in 50% formamide, 5 X Denhart's solution, 5 X SSPE (or SSC), 0.2% SDS at 42ºC (to about 50ºC), followed by washing in 0.2 X SSPE (or SSC) and 0.2% SDS at a temperature of at least about 42°C, preferably about 55°C, more preferably about 65°C.

Low stringency refers, by way of nonlimiting example, to conditions that permit hybridization in 10% formamide, 5 X Denhart's solution, 6 X SSPE (or SSC), 0.2% SDS at 42ºC, followed by washing in 1 X SSPE (or SSC) and 0.2% SDS at a temperature of about 45°C, preferably about 50°C.

For additional stringency conditions, see T. Maniatis et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring

Harbor, NY (1982). It is to be understood that the low, moderate and high stringency hybridization / washing conditions may be varied using a variety of ingredients, buffers and temperatures well known to and practiced by the skilled practitioner.

The terms complementary or complementarity refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, as well as in the design and use of PNA molecules.

The term homology refers to a degree of complementarity. There may be partial sequence homology or complete homology, wherein complete homology is equivalent to identity, e.g., 100% identity. A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (e.g., Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. Nonetheless, conditions of low stringency do not permit non-specific binding; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity). In the absence of non-

specific binding, the probe will not hybridize to the second non-complementary target sequence.

Those having skill in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on the CLUSTALW computer program (J.D. Thompson et al., 1994, *Nucleic Acids Research*, 2(22):4673-4680), or FASTDB, (Brutlag et al., 1990, *Comp. App. Biosci.*, 6:237-245), as known in the art. Although the FASTDB algorithm typically does not consider internal non-matching deletions or additions in sequences, i.e., gaps, in its calculation, this can be corrected manually to avoid an overestimation of the % identity. CLUSTALW, however, does take sequence gaps into account in its identity calculations.

Also available to those having skill in this art are the BLAST and BLAST 2.0 algorithms (Altschul et al., 1977, *Nucl. Acids Res.*, 25:3389-3402 and Altschul et al., 1990, *J. Mol. Biol.*, 215:403-410). The BLASTN program for nucleic acid sequences uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, and an expectation (E) of 10. The BLOSUM62 scoring matrix (Henikoff and Henikoff, 1989, *Proc. Natl. Acad. Sci., USA*, 89:10915) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

An HDAC polynucleotide of the present invention may show at least 27.7%, 35%, 40%, 44.1%, 48.2%, 50%, 55.4%, 58.6%, 59.8%, 60%, 60.2%, 67.8%, 70%, 80%, 81.5%, 85%, 90%, 91%, 92%, 93%, 94%, 94.2%, 94.4%, 95%, 96%, 97%, 97.2%, 97.5%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identity to a sequence provided in SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, SEQ ID NO:96, or a sequence complementary thereto. An HDAC polypeptide of the present invention may show at least 25%, 35%, 40%, 45%, 48.1%, 55.2%, 55.3%, 60%, 65%, 70%, 72%, 75%, 79%, 80%, 80.6%, 85%, 90%, 91%, 92%, 93%, 94%, 94.2%, 95%, 96%, 97%, 97.2%, 97.5%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%

identity to a sequence provided in any one of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, or SEQ ID NO:95.

In a preferred aspect of the invention, a HDAC polynucleotide shows at least 60.2%, 81.5%, or 94.4% identity to the HDAC9c nucleotide sequence (SEQ ID NO:88 or a sequence complementary thereto); or at least 27.7%, 48.2%, or 55.4% identity to the HDACX_v2 nucleotide sequence (SEQ ID NO:96 or a sequence complementary thereto). A HDAC polypeptide of the invention preferably shows at least 55.2%, 80.6%, or 94.2% identity to the HDAC9c amino acid sequence (SEQ ID NO:87); at least 55.3% identity to the HDACX_v2 amino acid sequence (SEQ ID NO:95); at least 72% identity to the amino acid sequence of BMY_HDAL1 (SEQ ID NO:2); at least 79% identity to the amino acid sequence of BMY_HDAL2 (SEQ ID NO:4); or at least 70% identity to the amino acid sequence of BMY_HDAL3 (SEQ ID NO:5).

A composition comprising a given polynucleotide sequence refers broadly to any composition containing the given polynucleotide sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising the polynucleotide sequences (e.g., SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96) encoding the novel HDAC polypeptides of this invention, or fragments thereof, or complementary sequences thereto, may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be in association with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be employed in an aqueous solution containing salts (e.g., NaCl), detergents or surfactants (e.g., SDS) and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, and the like).

The term "substantially purified" refers to nucleic acid sequences or amino acid sequences that are removed from their natural environment, i.e., isolated or separated by a variety of means, and are at least 60% free, preferably 75% to 85% free, and most preferably 90% or greater free from other components with which they are naturally associated.

The term sample, or biological sample, is meant to be interpreted in its broadest sense. A biological sample suspected of containing nucleic acid encoding an HDAC protein, or fragments thereof, or an HDAC protein itself, may comprise a body fluid, an extract from cells or tissue, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), organelle, or membrane isolated from a cell, a cell, nucleic acid such as genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for Northern analysis), cDNA (in solution or bound to a solid support), a tissue, a tissue print and the like.

Transformation refers to a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and partial bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. Transformed cells also include those cells that transiently express the inserted DNA or RNA for limited periods of time.

The term "mimetic" refers to a molecule, the structure of which is developed from knowledge of the structure of an HDAC protein, or portions thereof, and as such, is able to effect some or all of the actions of HDAC proteins.

The term "portion" with regard to a protein (as in "a portion of a given protein") refers to fragments or segments, for example, peptides, of that protein. The fragments may range in size from four or five amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein "comprising at least a portion of the amino acid sequence of the HDAC molecules presented herein can encompass a full-length human HDAC polypeptide, and fragments thereof.

In specific embodiments, HDAC polypeptides of the invention can comprise at least 5, 10, 20, 30, 50, 70, 100, 200, 300, 400, 500, 600, 700, 720, 750, 800, 920, or 950 contiguous amino acid residues of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, or SEQ ID NO:95. Additionally, a polypeptide of the invention can comprise a specific region, e.g., the C-terminal region, of a HDAC amino acid sequence. Such polypeptides can comprise, for example, amino acids 1009-1069 of HDAC9c (SEQ ID NO:87), amino acids 720-780 of HDACX_v1 (SEQ ID NO:93), or portions or fragments thereof.

The term antibody refers to intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, Fv, which are capable of binding an epitopic or antigenic determinant. Antibodies that bind to the HDAC polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest or prepared recombinantly for use as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g, a mouse, a rat, or a rabbit).

The term "humanized" antibody refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions, e.g., the complementarity determining regions (CDRs), in order to more closely resemble a human antibody, while still retaining the original binding capability, e.g., as described in U.S. Patent No. 5,585,089 to C.L. Queen et al., which is a nonlimiting example. Fully humanized antibodies, such as those produced transgenically or recombinantly, are also encompassed herein.

The term "antigenic determinant" refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein;

these regions or structures are referred to an antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding" refer to the interaction between a protein or peptide and a binding molecule, such as an agonist, an antagonist, or an antibody. The interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope, or a structural determinant) of the protein that is recognized by the binding molecule. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "correlates with expression of a polynucleotide" indicates that the detection of the presence of ribonucleic acid that is similar to one or more of the HDAC sequences provided herein by Northern analysis is indicative of the presence of mRNA encoding an HDAC polypeptide in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

An alteration in the polynucleotide of an HDAC nucleic acid sequence comprises any alteration in the sequence of the polynucleotides encoding an HDAC polypeptide, including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes an HDAC polypeptide (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to the HDAC nucleic acid sequences presented herein, (i.e., SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and/or SEQ ID NO:96), the inability of a selected fragment of a given HDAC sequence to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding

an HDAC polypeptide (e.g., using fluorescent *in situ* hybridization (FISH) to metaphase chromosome spreads).

Description of Embodiments of the Present Invention

In one of its embodiments, the present invention is directed to a novel HDAC termed, BMY_HDAL1, which is encoded by the human BAC clones AC016186, AC00755 and AC002088. The BMY_HDAL1 nucleic acid (cDNA) sequence is provided as SEQ ID NO:1; the BMY_HDAL1 amino acid sequence encoded by the BMY_HDAL1 nucleic acid sequence is presented as SEQ ID NO:2. (FIG. 1).

BMY_HDAL1 was identified by HMM analysis using PFAM model PF00850. (Example 1). The PFAM-HMM database is a collection of protein families and domains and contains multiple protein alignments (A. Bateman et al., 1999, *Nucleic Acids Research*, 27:260-262). BMY_HDAL1 is most closely related to the known human histone deacetylase HDAC5; the two proteins are 71% identical and 77% similar over 105 amino acids, as determined by the GCG Gap program with a gap weight of 8 and a length weight of 2. The gene structure and predicted cDNA and protein sequence of BMY_HDAL1 were determined by comparison to the known human histone deacetylase HDAC5 using the GenewiseDB program to analyze human BAC AC002088 (E. Birney and R. Durbin, 2000, *Genome Res.*, 10(4):547-548).

Sequence motifs of BMY_HDAL1 were examined using the GCG Motifs program to ascertain if there were motifs common to other known proteins in the PROSITE collection (K. Hofmann et al., 1999, *Nucleic Acids Res.*, 27(1):215-219) with no allowed mismatches. Motifs programs typically search for protein motifs by searching protein sequences for regular-expression patterns described in the PROSITE Dictionary. FIG. 4 shows PROSITE motifs identified in the partial predicted amino acid sequence of BMY_HDAL1.

In another embodiment, the present invention is directed to the novel HDAC termed BMY_HDAL2, a novel human histone deacetylase-like protein encoded by genomic BACs AC002410. The BMY_HDAL2 nucleic acid sequence (SEQ ID NO:12) and its encoded polypeptide (SEQ ID NO:4) are

presented in FIG. 5. BMY_HDAL2 was identified by hidden Markov model searches using the PFAM HMM PF00850 to search predicted proteins from human genomic DNA. BMY_HDAL2 is most closely related to the known human histone deacetylase HDAC5; the two proteins are 78% identical and 86% similar over 163 amino acids as determined by the GCG Gap program with a gap weight of 8 and a length weight of 2. The gene structure and predicted cDNA and protein sequences of BMY_HDAL2 were determined by comparison to BMY_HDA5 using the GenewiseDB program (E. Birney and R. Durbin, 2000, *Genome Res.*, 10(4):547-548).

Sequence motifs of BMY_HDAL2 were examined using the GCG Motifs program to ascertain if there were motifs in the PROSITE collection (K. Hofmann et al., 1999, *Nucleic Acids Res.*, 27(1):215-219) with no allowed mismatches. FIG. 7 shows PROSITE motifs identified in the partial predicted amino acid sequence of BMY_HDAL2.

In addition, the genomic location surrounding BMY_HDAL2 was investigated. Based on the genomic location of BAC AC002410 as reported by the NCBI MapViewer, BMY_HDAL2 has been localized to chromosome 7 region q36.

In another embodiment, the present invention further provides a third HDAC termed BMY_HDAL3. The BMY_HDAL3 nucleic acid sequence (SEQ ID NO:19) and its encoded polypeptide (SEQ ID NO:5) are presented in FIG. 10. BMY_HDAL3 is encoded by the human genomic BAC clones AC004994 and AC004744. BMY_HDAL3 was identified by HMM analysis using PFAM model PF00850 to search predicted proteins generated from human genomic DNA sequences using Genscan. BMY_HDAL3 is most closely related to the known human histone deacetylase HDAC5; the two proteins are 69% identical over 1122 amino acids as determined by the GCG Gap program with a gap weight of 8 and a length weight of 2.

The partial transcripts identified from BAC clones AC004994 (SEQ ID NO:15) and AC004744 (SEQ ID NO:16) were assembled into a single contig (designated BMY_HDAL3) using the VectorNTI ContigExpress program (Informax). (FIG. 9). The gene structure and predicted cDNA and protein

sequence of BMY_HDAL3 were determined by comparison to the known human histone deacetylase HDAC5 using the GenewiseDB program (K. Hofmann et al., 1999, *Nucleic Acids Res.*, 27(1):215-219) and are presented in FIG. 9. The most N-terminal region of the BMY_HDAL3 sequence described herein is encoded by human genomic BAC AC004994. (FIG. 8A).

BMY_HDAL3 has been localized to chromosome 7, region q36 based on the locations reported for AC004994 and by the NCBI MapViewer.

Sequence motifs of BMY_HDAL3 were examined using the GCG Motifs program to ascertain if there were motifs in the PROSITE collection (K. Hofmann et al., 1999, *Nucleic Acids Res.*, 27(1):215-219) with no allowed mismatches. FIG. 11 shows PROSITE motifs identified in the partial predicted amino acid sequence of BMY_HDAL3. FIG. 12 shows a multiple sequence alignment of the novel human HDAC, BMY_HDAL3, amino acid sequence (SEQ ID NO:5) with the amino acid sequence of AAC78618 (SEQ ID NO:21) and with the amino acid sequence of AAD15364 (SEQ ID NO:22). AAC78618 is a histone deacetylase-like protein predicted by genefinding and conceptual translation of AC004994 and which was entered in Genbank. AAD15364 is a similar predicted protein derived from AC004744 and entered in Genbank. AAC78618, AAD15364 and BMY_HDAL3 were aligned using the ClustalW algorithm as implemented in the VectorNTI sequence analysis package (1998, 5.5 Ed., Informax, Inc.) with a gap opening penalty of 10, a gap extension penalty of 0.1 and no end gap penalties.

Novel HDAC9 variants, termed HDACX_v1 and HDACX_v2, have also been identified. In addition, HDAC9c, an HDAC9-related family member, has been newly identified and cloned.

HDAC Polynucleotides and Polypeptides

The present invention encompasses novel HDAC nucleic acid sequences (e.g., SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, SEQ ID NO:96, and sequences complementary thereto) encoding newly discovered histone deacetylase like polypeptides (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95). These HDAC polynucleotides, polypeptides, or

compositions thereof, can be used in methods for screening for antagonists or inhibitors of the activity or function of HDACs.

In another of its embodiments, the present invention encompasses new HDAC polypeptides comprising the amino acid sequences of, e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95, and as shown in FIG. 1, FIG. 5, FIG. 10, FIGS. 15A-15C, FIGS. 20A-20C, and FIGS. 21A-21B.

The HDAC polypeptides as described herein show close similarity to HDAC proteins, including HDAC5 and HDAC9. FIGS. 2A and 2B portray the structural similarities among the novel HDAC polypeptides and several other proteins, namely Aquifex HDAL, Human HDAC4, Human HDAC5, Human HDAC7, and *Saccharomyces cerevisiae* HDA1. FIGS. 15D-15F show the amino acid sequence similarity and identity shared by HDAC9c and previously identified HDAC9 amino acid sequences. FIGS. 23A-23K show the nucleotide sequence identity shared by HDACX_v1, HDACX_v2, and previously identified HDAC9 nucleotide sequences.

Variants of the disclosed HDAC polynucleotides and polypeptides are also encompassed by the present invention. In some cases, a HDAC polynucleotide variant (i.e., variant of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96) will encode an amino acid sequence identical to a HDAC sequence (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95). This is due to the redundancy (degeneracy) of the genetic code, which allows for silent mutations. In other cases, a HDAC polynucleotide variant will encode a HDAC polypeptide variant (i.e., a variant of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, or SEQ ID NO:95). Preferably, an HDAC polypeptide variant has at least 75 to 80%, more preferably at least 85 to 90%, and even more preferably at least 90% or greater amino acid sequence identity to one or more of the HDAC amino acid sequences (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95) as disclosed herein, and which retains at least one biological or other functional characteristic or activity of the HDAC

polypeptide. Most preferred is a variant having at least 95% amino acid sequence identity to the amino acid sequences set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95.

An amino acid sequence variant of the HDAC proteins can be categorized into one or more of three classes: substitutional, insertional, or deletional variants. Such variants are typically prepared by site-specific mutagenesis of nucleotides in the DNA encoding the HDAC protein, using cassette or PCR mutagenesis, or other techniques that are well known and practiced in the art, to produce DNA encoding the variant. Thereafter, the DNA is expressed in recombinant cell culture as described herein. Variant HDAC protein fragments having up to about 100-150 residues may be prepared by in vitro synthesis using conventional techniques.

Amino acid sequence variants are characterized by the predetermined nature of the variation, a feature that sets them apart from naturally occurring allelic or interspecies variations of an HDAC amino acid sequence. The variants typically exhibit the same qualitative biological activity as that of the naturally occurring analogue, although variants can also be selected having modified characteristics. While the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be performed at the target codon or region, and the expressed HDAC variants can be screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is accomplished using assays of HDAC protein activity, for example, for binding domain mutations, competitive binding studies may be carried out.

Amino acid substitutions are typically of single residues; insertions usually are on the order of from one to twenty amino acids, although considerably larger insertions may be tolerated. Deletions range from about

one to about 20 residues, although in some cases, deletions may be much larger.

Substitutions, deletions, insertions, or any combination thereof, may be used to arrive at a final HDAC derivative. Generally, these changes affect only a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances. When small alterations in the characteristics of the HDAC protein are desired or warranted, substitutions are generally made in accordance with the following table:

| Original Residue | Conservative Substitution(s) | Original Residue | Conservative Substitution(s) |
|---|---|---|---|
| Ala | Ser | Leu | Ile, Val |
| Arg | Lys | Lys | Arg, Gln, Glu |
| Asn | Gln, His | Met | Leu, Ile |
| Asp | Glu | Phe | Met, Leu, Tyr |
| Cys | Ser | Ser | Thr |
| Gln | Asn | Thr | Ser |
| Glu | Asp | Trp | Tyr |
| Gly | Pro | Tyr | Trp, Phe |
| His | Asn, Gln | Val | Ile, Leu |
| Ile | Leu, Val | | |

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in the above Table. For example, substitutions may be made which more significantly affect the structure of the polypeptide backbone in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which generally are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue

having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

While HDAC variants will ordinarily exhibit the same qualitative biological activity or function, and elicit the same immune response, as the naturally occurring analogue, the variants are also selected to modify the characteristics of HDAC proteins as needed. Alternatively, the variant may be designed such the that biological activity of the HDAC protein is altered, e.g., improved.

In another embodiment, the present invention encompasses polynucleotides that encode the novel HDAC polypeptides disclosed herein. Accordingly, any nucleic acid sequence that encodes the amino acid sequence of an HDAC polypeptide of the invention can be used to produce recombinant molecules that express that HDAC protein. In a particular embodiment, the present invention encompasses the novel human HDAC polynucleotides comprising the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and SEQ ID NO:96 as shown in FIG. 1, FIG. 5, FIG. 10, FIGS. 15A-15C, FIGS. 20A-20C, and FIGS. 21A-21B. More particularly, the present invention embraces cloned full-length open reading frame human BMY_HDAL1, BMY_HDAL2 and BMY_HDAL3 deposited at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 on _____ under ATCC Accession No. _____ _____ according to the terms of the Budapest Treaty.

As will be appreciated by the skilled practitioner in the art, the degeneracy of the genetic code results in the production of more than one appropriate nucleotide sequence encoding the HDAC polypeptides of the present invention. Some of the sequences bear minimal homology to the nucleotide sequences of any known and naturally occurring gene. Accordingly, the present invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are

made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of a naturally occurring HDAC protein, and all such variations are to be considered as being embraced herein.

Although nucleotide sequences which encode the HDAC polypeptides and variants thereof are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HDAC polypeptides under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding the HDAC polypeptides, or derivatives thereof, which possess a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide/polypeptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host, for example, in plant cells or yeast cells or amphibian cells. Other reasons for substantially altering the nucleotide sequence encoding the HDAC polypeptides, and derivatives, without altering the encoded amino acid sequences, include the production of mRNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The present invention also encompasses production of DNA sequences, or portions thereof, which encode the HDAC polypeptides, and derivatives of these polypeptides, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known and practiced by those in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding an HDAC polypeptide, or any fragment thereof.

Also encompassed by the present invention are polynucleotide sequences that are capable of hybridizing to the HDAC nucleotide sequences presented herein, such as those shown in SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and SEQ ID NO:96, or sequences complementary thereto, under various conditions of stringency. Hybridization conditions are typically based on the melting temperature (Tm) of the nucleic

acid binding complex or probe (See, G.M. Wahl and S.L. Berger, 1987; *Methods Enzymol.*, 152:399-407 and A.R. Kimmel, 1987; *Methods of Enzymol.*, 152:507-511), and may be used at a defined stringency. For example, included in the present invention are sequences capable of hybridizing under moderately stringent conditions to the HDAC nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:12, or SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and SEQ ID NO:96, and other sequences which are degenerate to those which encode the HDAC polypeptides (e.g., as a nonlimiting example: prewashing solution of 2 X SSC, 0.5% SDS, 1.0mM EDTA, pH 8.0, and hybridization conditions of 50°C, 5 X SSC, overnight).

In another embodiment of the present invention, polynucleotide sequences or fragments (peptides) thereof which encode the HDAC polypeptide may be used in recombinant DNA molecules to direct the expression of the HDAC polypeptide products, or fragments or functional equivalents thereof, in appropriate host cells. Because of the inherent degeneracy of the genetic code, other DNA sequences, which encode substantially the same or a functionally equivalent amino acid sequences, may be produced, and these sequences may be used to express recombinant HDAC polypeptides.

As will be appreciated by those having skill in the art, it may be advantageous to produce HDAC polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HDAC polypeptide-encoding sequences for a variety of reasons, including, but not limited to, alterations which modify the cloning, processing, and/or expression of the gene products. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer

the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and the like.

In another embodiment of the present invention, natural, modified, or recombinant nucleic acid sequences, or a fragment thereof, encoding the HDAC polypeptides may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening peptide libraries for inhibitors or modulators of HDAC activity or binding, it may be useful to encode a chimeric HDAC protein or peptide that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between an HDAC protein-encoding sequence and the heterologous protein sequence, so that the HDAC protein may be cleaved and purified away from the heterologous moiety.

In another embodiment, ligand-binding assays are useful to identify inhibitor or antagonist compounds that interfere with the function of the HDAC protein, or activator compounds that stimulate the function of the HDAC protein. Preferred are inhibitor or antagonist compounds. Such assays are useful even if the function of a protein is not known. These assays are designed to detect binding of test compounds (i.e., test agents) to particular target molecules, e.g., proteins or peptides. The detection may involve direct measurement of binding. Alternatively, indirect indications of binding may involve stabilization of protein structure, or disruption or enhancement of a biological function. Non-limiting examples of useful ligand-binding assays are detailed below.

One useful method for the detection and isolation of binding proteins is the Biomolecular Interaction Assay (BIAcore) system developed by Pharmacia Biosensor and described in the manufacturer's protocol (LKB Pharmacia, Sweden). The BIAcore system uses an affinity purified anti-GST antibody to immobilize GST-fusion proteins onto a sensor chip. The sensor utilizes surface plasmon resonance, which is an optical phenomenon that detects changes in refractive indices. Accordingly, a protein of interest, e.g., an HDAC polypeptide, or fragment thereof, of the present invention, is coated

onto a chip and test compounds (i.e., test agents) are passed over the chip. Binding is detected by a change in the refractive index (surface plasmon resonance).

A different type of ligand-binding assay involves scintillation proximity assays (SPA), as described in U.S. Patent No. 4,568,649. In a modification of this assay currently undergoing development, chaperonins are used to distinguish folded and unfolded proteins. A tagged protein is attached to SPA beads, and test compounds are added. The bead is then subjected to mild denaturing conditions, such as, for example, heat, exposure to SDS, and the like, and a purified labeled chaperonin is added. If a test compound (i.e., test agent) has bound to a target protein, the labeled chaperonin will not bind; conversely, if no test compound has bound, the protein will undergo some degree of denaturation and the chaperonin will bind. In another type of ligand binding assay, proteins containing mitochondrial targeting signals are imported into isolated mitochondria *in vitro* (Hurt et al., 1985, *EMBO J.*, 4:2061-2068; Eilers and Schatz, 1986, *Nature*, 322:228-231).

In a mitochondrial import assay, expression vectors are constructed in which nucleic acids encoding particular target proteins are inserted downstream of sequences encoding mitochondrial import signals. The chimeric proteins are synthesized and tested for their ability to be imported into isolated mitochondria in the absence and presence of test compounds. A test compound that binds to the target protein should inhibit its uptake into isolated mitochondria *in vitro*.

Another type of ligand-binding assay suitable for use according to the present invention is the yeast two-hybrid system (Fields and Song, 1989, *Nature*, 340:245-246). The yeast two-hybrid system takes advantage of the properties of the GAL4 protein of the yeast *S. cerevisiae*. The GAL4 protein is a transcriptional activator required for the expression of genes encoding enzymes involving the utilization of galactose. GAL4 protein consists of two separable and functionally essential domains: an N-terminal domain, which binds to specific DNA sequences (UASG); and a C-terminal domain containing acidic regions, which is necessary to activate transcription. The

native GAL4 protein, containing both domains, is a potent activator of transcription when yeast cells are grown on galactose medium. The N-terminal domain binds to DNA in a sequence-specific manner but is unable to activate transcription. The C-terminal domain contains the activating regions but cannot activate transcription because it fails to be localized to UASG. In the two-hybrid system, a system of two hybrid proteins containing parts of GAL4: (1) a GAL4 DNA-binding domain fused to a protein 'X', and (2) a GAL4 activation region fused to a protein 'Y'. If X and Y can form a protein-protein complex and reconstitute proximity of the GAL4 domains, transcription of a gene regulated by UASG occurs. Creation of two hybrid proteins, each containing one of the interacting proteins X and Y, allows the activation region of UASG to be brought to its normal site of action.

The binding assay described in Fodor et al., 1991, *Science*, 251:767-773, which involves testing the binding affinity of test compounds for a plurality of defined polymers synthesized on a solid substrate, may also be useful. Compounds that bind to an HDAC polypeptide, or portions thereof, according to this invention are potentially useful as agents for use in therapeutic compositions.

In another embodiment, sequences encoding an HDAC polypeptide may be synthesized in whole, or in part, using chemical methods well known in the art (See, for example, M.H. Caruthers et al., 1980, *Nucl. Acids Res. Symp. Ser.*, 215-223 and T. Horn, T et al., 1980, *Nucl. Acids Res. Symp. Ser.*, 225-232). Alternatively, an HDAC protein or peptide itself may be produced using chemical methods to synthesize the amino acid sequence of the HDAC polypeptide or peptide, or a fragment or portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (J.Y. Roberge et al., 1995, *Science*, 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (PE Biosystems).

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (e.g., T. Creighton, 1983, *Proteins, Structures and Molecular Principles,* WH Freeman and Co., New

York, N.Y), by reversed-phase high performance liquid chromatography, or other purification methods as are known in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, *supra*). In addition, the amino acid sequence of an HDAC polypeptide, peptide, or any portion thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

Expression of Human HDAC Proteins

To express a biologically active / functional HDAC polypeptide or peptide, the nucleotide sequences encoding the HDAC polypeptides, or functional equivalents, may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods that are well known to and practiced by those skilled in the art may be used to construct expression vectors containing sequences encoding an HDAC polypeptide or peptide and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in J. Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y. and in F.M. Ausubel et al., 1989, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding an HDAC polypeptide or peptide. Such expression vector/host systems include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast or fungi transformed with yeast or fungal expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)), or with bacterial expression vectors

(e.g., Ti or pBR322 plasmids); or animal cell systems. The host cell employed is not limiting to the present invention.

"Control elements" or "regulatory sequences" are those non-translated regions of the vector, e.g., enhancers, promoters, 5' and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, La Jolla, CA) or PSPORT1 plasmid (Life Technologies), and the like, may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes), or from plant viruses (e.g., viral promoters or leader sequences), may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding an HDAC polypeptide or peptide, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected, depending upon the use intended for the expressed HDAC product. For example, when large quantities of expressed protein are needed for the induction of antibodies, vectors that direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding an HDAC polypeptide, or peptide, may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase, so that a hybrid protein is produced; pIN vectors (See, G. Van Heeke and S.M. Schuster, 1989, *J. Biol. Chem.*, 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign

polypeptides, as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can be easily purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. (For reviews, see F.M. Ausubel et al., *supra*, and Grant et al., 1987, *Methods Enzymol.*, 153:516-544).

Should plant expression vectors be desired and used, the expression of sequences encoding an HDAC polypeptide or peptide may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (N. Takamatsu, 1987, *EMBO J.*, 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO, or heat shock promoters, may be used (G. Coruzzi et al., 1984, *EMBO J.*, 3:1671-1680; R. Broglie et al., 1984, *Science*, 224:838-843; and J. Winter et al., 1991, *Results Probl. Cell Differ.* 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (See, for example, S. Hobbs or L.E. Murry, In: McGraw Hill *Yearbook of Science and Technology* (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express an HDAC polypeptide or peptide. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequences encoding an HDAC polypeptide or peptide may be cloned into a non-essential region of the virus such as the polyhedrin gene and placed under control of the polyhedrin promoter. Successful insertion of the HDAC polypeptide or

peptide will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia* larvae in which the HDAC polypeptide or peptide product may be expressed (E.K. Engelhard et al., 1994, *Proc. Nat. Acad. Sci.*, 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding an HDAC polypeptide or peptide may be ligated into an adenovirus transcription/translation complex containing the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the HDAC polypeptide or peptide in infected host cells (J. Logan and T. Shenk, 1984, *Proc. Natl. Acad. Sci.*, 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding an HDC polypeptide or peptide. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding an HDAC polypeptide or peptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals, including the ATG initiation codon, should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system that is used, such as those described in the literature (D. Scharf et al., 1994, *Results Probl. Cell Differ.*, 20:125-162).

Moreover, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein

in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells having specific cellular machinery and characteristic mechanisms for such post-translational activities (e.g., COS, CHO, HeLa, MDCK, HEK293, and W138) are available from the American Type Culture Collection (ATCC), American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, and may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express an HDAC protein may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same, or on a separate, vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched cell culture medium before they are switched to selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows the growth and recovery of cells that successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the Herpes Simplex Virus thymidine kinase (HSV TK), (M. Wigler et al., 1977, *Cell*, 11:223-32) and adenine phosphoribosyltransferase (I. Lowy et al., 1980, *Cell*, 22:817-23) genes which can be employed in $tk^-$ or $aprt^-$ cells, respectively. Also, anti-metabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr, which confers resistance to methotrexate (M. Wigler et al., 1980, *Proc. Natl. Acad. Sci.*, 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (F. Colbere-Garapin

et al., 1981, *J. Mol. Biol.*, 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (S.C. Hartman and R.C. Mulligan, 1988, *Proc. Natl. Acad. Sci.*, 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as the anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, which are widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression that is attributable to a specific vector system (C.A. Rhodes et al., 1995, *Methods Mol. Biol.*, 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the desired gene of interest may need to be confirmed. For example, if an HDAC nucleic acid sequence is inserted within a marker gene sequence, recombinant cells containing sequences encoding the HDAC polypeptide or peptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding an HDAC polypeptide or peptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates co-expression of the tandem gene.

Alternatively, host cells which contain the nucleic acid sequence encoding an HDAC polypeptide or peptide and which express the HDAC product may be identified by a variety of procedures known to those having skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques, including membrane, solution, or chip based technologies, for the detection and/or quantification of nucleic acid or protein.

Preferably, the HDAC polypeptide or peptide of this invention is substantially purified after expression. HDAC proteins and peptides can be isolated or purified in a variety of ways known to and practiced by those

having skill in the art, depending on what other components may be present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including, but not limited to, ion exchange, hydrophobic affinity and reverse phase HPLC chromatography, and chromatofocusing. For example, an HDAC protein or peptide can be purified using a standard anti-HDAC antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see R. Scopes, 1982, *Protein Purification*, Springer-Verlag, NY. As will be understood by the skilled practitioner, the degree of purification necessary will vary depending on the intended use of the HDAC protein or peptide; in some instances, no purification will be necessary.

In addition to recombinant production, fragments of an HDAC polypeptide or peptide may be produced by direct peptide synthesis using solid-phase techniques (J. Merrifield, 1963, *J. Am. Chem. Soc.*, 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using ABI 431A Peptide Synthesizer (PE Biosystems). If desired, various fragments of an HDAC polypeptide can be chemically synthesized separately and then combined using chemical methods to produce the full length molecule.

Detection of Human HDAC Polynucleotide

The presence of polynucleotide sequences encoding an HDAC polypeptide or this invention can be detected by DNA-DNA or DNA-RNA hybridization, or by amplification using probes or portions or fragments of polynucleotides encoding the HDAC polypeptide. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers, based on the sequences encoding a particular HDAC polypeptide or peptide, to detect transformants containing DNA or RNA encoding an HDAC polypeptide or peptide.

A wide variety of labels and conjugation techniques are known and employed by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR

probes for detecting sequences related to polynucleotides encoding an HDAC polypeptide or peptide include oligo-labeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding an HDAC polypeptide, or any portions or fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase, such as T7, T3, or SP(6) and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (e.g., Amersham Pharmacia Biotech, Promega and U.S. Biochemical Corp.).

Suitable reporter molecules or labels which may be used include radionucleotides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like. Non-limiting examples of labels include radioisotopes, such as $^{3}H$, $^{14}C$, and $^{32}P$, and non-radioactive molecules, such as digoxigenin. In addition, nucleic acid molecules may be modified using known techniques, for example, using RNA or DNA analogs, phosphorylation, dephosphorylation, methylation, or demethylation.

Human HDAC Polypeptides – Production, Detection, Isolation

Host cells transformed with nucleotide sequences encoding an HDAC protein or peptide, or fragments thereof, may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those having skill in the art, expression vectors containing polynucleotides which encode an HDAC protein or peptide may be designed to contain signal sequences that direct secretion of the HDAC protein or peptide through a prokaryotic or eukaryotic cell membrane.

Other constructions may be used to join nucleic acid sequences encoding an HDAC protein or peptide to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating

peptides such as histidine-tryptophan modules that allow purification on immobilized metals; protein A domains that allow purification on immobilized immunoglobulin; and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the HDAC protein or peptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing HDAC-encoding sequence and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography) as described by J. Porath et al., 1992, *Prot. Exp. Purif.*, 3:263-281, while the enterokinase cleavage site provides a means for purifying from the fusion protein. For a discussion of suitable vectors for fusion protein production, see D.J. Kroll et al., 1993; *DNA Cell Biol.*, 12:441-453.

Human artificial chromosomes (HACs) may be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid vector. HACs are linear microchromosomes which may contain DNA sequences of 10K to 10M in size, and contain all of the elements that are required for stable mitotic chromosome segregation and maintenance (See, J.J. Harrington et al., 1997, *Nature Genet.*, 15:345-355). HACs of 6 to 10M are constructed and delivered via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

A variety of protocols for detecting and measuring the expression of an HDAC polypeptide using either polyclonal or monoclonal antibodies specific for the protein are known and practiced in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive with two non-interfering epitopes on the HDAC polypeptide is preferred, but a competitive binding assay may also be employed. These and other assays are described in the art as represented by the publication of R. Hampton et al., 1990; *Serological*

*Methods, a Laboratory Manual,* APS Press, St Paul, MN and D.E. Maddox et al., 1983; *J. Exp. Med.*, 158:1211-1216).

For use with these assays, amino acid sequences (e.g., polypeptides, peptides, antibodies, or antibody fragments) may be attached to a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotope, fluorescent, and enzyme labels. Fluorescent labels include, for example, Cy3, Cy5, Alexa, BODIPY, fluorescein (e.g., FluorX, DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Preferred isotope labels include $^{3}H$, $^{14}C$, $^{32}P$, $^{35}S$, $^{36}Cl$, $^{51}Cr$, $^{57}Co$, $^{58}Co$, $^{59}Fe$, $^{90}Y$, $^{125}I$, $^{131}I$, and $^{186}Re$. Preferred enzyme labels include peroxidase, β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, glucose oxidase plus peroxidase, and alkaline phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSA™), are known in the art, and are commercially available (see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, CA; NEN® Life Science Products, Inc., Boston, MA).

A compound that interacts with a histone deacetylase according to the present invention may be one that is a substrate for the enzyme, one that binds the enzyme at its active site, or one that otherwise acts to alter enzyme activity by binding to an alternate site. A substrate may be acetylated histones, or a labeled acetylated peptide fragment derived therefrom, such as AcGly-Ala-Lys,(.epsilon.-Ac)-Arg-His-Arg-Lys,(.epsilon.-Ac)-Val$NH_2$, or other synthetic or naturally occurring substrates. Examples of compounds that bind to histone deacetylase are known inhibitors such as n-butyrate, trichostatin, trapoxin and SAHA (S. Swendeman et al., 1999, *Cancer Res.*, 59(17):4392-4399). The compound that interacts with a histone deacetylase is preferably

labeled to allow easy quantification of the level of interaction between the compound and the enzyme. A preferred radiolabel is tritium.

The test compound (i.e., test agent) may be a synthetic compound, a purified preparation, crude preparation, or an initial extract of a natural product obtained from plant, microorganism or animal sources.

One aspect of the present method is based on test compound- induced inhibition of histone deacetylase activity. The enzyme inhibition assay involves adding histone deacetylase or an extract containing histone deacetylase to mixtures of an enzyme substrate and the test compound, both of which are present in known concentrations. The amount of the enzyme is chosen such that approximately 20% of the substrate is consumed during the assay. The assay is carried out with the test compound at a series of different dilution levels. After a period of incubation, the labeled portion of the substrate released by enzymatic action is separated and counted. The assay is generally carried out in parallel with a negative control (i.e., no test compound) and a positive control (i.e., containing a known enzyme inhibitor instead of a test compound). The concentration of the test compound at which 50% of the enzyme activity is inhibited ($IC_{50}$) is determined using art recognized method.

Although enzyme inhibition is the most direct measure of the inhibitory activity of the test compound, results obtained from a competitive binding assay in which the test compound competes with a known inhibitor for binding to the enzyme active site correlate well with the results obtained from enzyme inhibition assay described above. The binding assay represents a more convenient way to assess enzyme inhibition, because it allows the use of a crude extract containing histone deacetylase rather than partially purified enzyme. The use of a crude extract may not always be suitable in the enzyme inhibition assay because other enzymes present in the extract may act on the histone deacetylase substrate.

The competition binding assay is carried out by adding a histone deacetylase, or an extract containing histone deacetylase activity, to a mixture of the test compound and a labeled inhibitor, both of which are present in the

mixture in known concentrations. After incubation, the enzyme-inhibitor complex is separated from the unbound labeled inhibitors and unlabeled test compound, and counted. The concentration of the test compound required to inhibit 50% of the binding of the labeled inhibitor to the histone deacetylase ($IC_{50}$) is calculated.

In one method suitable for this invention, the $IC_{50}$ of test compounds against host histone deacetylase is determined using either the enzyme inhibition assay or the binding assay as described above, to identify those compounds that have selectivity for a particular type of histone deacetylase over that of a host.

Anti-Human HDAC Antibodies and Uses Thereof

Antagonists or inhibitors of the HDAC polypeptides of the present invention may be produced using methods that are generally known in the art. In particular, purified HDAC polypeptides or peptides, or fragments thereof, can be used to produce antibodies, or to screen libraries of pharmaceutical agents or other compounds, particularly, small molecules, to identify those which specifically bind to the novel HDACs of this invention.

Antibodies specific for an HDAC polypeptide, or immunogenic peptide fragments thereof, can be generated using methods that have long been known and conventionally practiced in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by an Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, sheep, rats, mice, humans, and others, can be immunized by injection with HDAC polypeptide, or any peptide fragment or oligopeptide thereof, which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase the immunological response. Nonlimiting examples of suitable adjuvants include Freund's (incomplete), mineral gels such as aluminum hydroxide or silica, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and

dinitrophenol. Adjuvants typically used in humans include BCG (bacilli Calmette Guérin) and *Corynebacterium parvumn.*

Preferably, the peptides, fragments, or oligopeptides used to induce antibodies to HDAC polypeptides (i.e., immunogens) have an amino acid sequence having at least five amino acids, and more preferably, at least 7-10 amino acids. It is also preferable that the immunogens are identical to a portion of the amino acid sequence of the natural protein; they may also contain the entire amino acid sequence of a small, naturally occurring molecule. The peptides, fragments or oligopeptides may comprise a single epitope or antigenic determinant or multiple epitopes. Short stretches of HDAC amino acids may be fused with those of another protein, such as KLH, and antibodies are produced against the chimeric molecule.

Monoclonal antibodies to HDAC polypeptides, or immunogenic fragments thereof, may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (G. Kohler et al., 1975, *Nature*, 256:495-497; D. Kozbor et al., 1985, *J. Immunol. Methods*, 81:31-42; R.J. Cote et al., 1983, *Proc. Natl. Acad. Sci. USA*, 80:2026-2030; and S.P. Cole et al., 1984, *Mol. Cell Biol.*, 62:109-120). The production of monoclonal antibodies is well known and routinely used in the art.

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (S.L. Morrison et al., 1984, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855; M.S. Neuberger et al., 1984, *Nature*, 312:604-608; and S. Takeda et al., 1985, *Nature*, 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HDAC polypeptide- or peptide-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (D.R. Burton, 1991, *Proc. Natl. Acad. Sci. USA*,

88:11120-3). Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (R. Orlandi et al., 1989, *Proc. Natl. Acad. Sci. USA*, 86:3833-3837 and G. Winter et al., 1991, *Nature*, 349:293-299).

Antibody fragments that contain specific binding sites for an HDAC polypeptide or peptide may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (W.D. Huse et al., 1989, *Science*, 254.1275-1281).

Various immunoassays can be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve measuring the formation of complexes between an HDAC polypeptide and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive with two non-interfering HDAC epitopes is preferred, but a competitive binding assay may also be employed (Maddox, *supra*).

Antibodies which specifically bind HDAC epitopes can also be used in immunohistochemical staining of tissue samples to evaluate the abundance and pattern of expression of each of the provided HDAC polypeptides. Anti-HDAC antibodies can be used diagnostically in immuno-precipitation and immunoblotting techniques to detect and evaluate HDAC protein levels in tissue as part of a clinical testing procedure. For instance, such measurements can be useful in predictive evaluations of the onset or progression of proliferative or differentiation disorders. Similarly, the ability to monitor HDAC protein levels in an individual can allow the determination of the efficacy of a given treatment regimen for an individual afflicted with such a

disorder. The level of HDAC polypeptide may be measured from cells in a bodily fluid, such as in samples of cerebral spinal fluid or amniotic fluid, or can be measured in tissue, such as produced by biopsy. Diagnostic assays using anti-HDAC antibodies can include, for example, immunoassays designed to aid in early diagnosis of a disorder, particularly ones that are manifest at birth. Diagnostic assays using anti-HDAC polypeptide antibodies can also include immunoassays designed to aid in early diagnosis and phenotyping of neoplastic or hyperplastic disorders.

Another application of anti-HDAC antibodies according to the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as λgt11, λgt 18-23, λZAP, and λORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins. For example, λgt11 will produce fusion proteins whose amino termini contain 13-galactosidase amino acid sequences and whose carboxy termini contain a foreign polypeptide. Antigenic epitopes of an HDAC protein, e.g. other orthologs of a particular HDAC protein or other paralogs from the same species, can then be detected with antibodies by, for example, reacting nitrocellulose filters lifted from infected plates with anti-HDAC antibodies. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of HDAC homologs can be detected and cloned from other animals, as can alternative isoforms (including splice variants) from humans.

Therapeutics/Treatments/Methods of Use Involving HDACs

In an embodiment of the present invention, the polynucleotide encoding an HDAC polypeptide or peptide, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding a novel HDAC polypeptide may be used in situations in which it would be desirable to block the transcription of HDAC mRNA. In particular, cells may be transformed or transfected with sequences complementary to polynucleotides encoding an HDAC polypeptide. Thus, complementary molecules may be used to modulate human HDAC polynucleotide and polypeptide activity, or to achieve regulation of gene

function. Such technology is now well known in the art, and sense or antisense oligomers or oligonucleotides, or larger fragments, can be designed from various locations along the coding or control regions of polynucleotide sequences encoding the HDAC polypeptides. For antisense therapeutics, the oligonucleotides in accordance with this invention preferably comprise at least 3 to 50 nucleotides of a sequence complementary to SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96. It is more preferred that such oligonucleotides and analogs comprise at least 8 to 25 nucleotides, and still more preferred to comprise at least 12 to 20 nucleotides of this sequence.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express nucleic acid sequences that are complementary to the nucleic acid sequences encoding the novel HDAC polypeptides and peptides of the present invention. These techniques are described both in J. Sambrook et al., *supra* and in F.M. Ausubel et al., *supra*.

A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, e.g. a cDNA encoding the particular HDAC polypeptide desired. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. In addition, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid. As mentioned, retrovirus vectors, adenovirus vectors and adeno-associated virus vectors are exemplary recombinant gene delivery system for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host.

In addition to the above-illustrated viral transfer methods, non-viral methods can also be employed to yield expression of an HDAC polypeptide in

the cells and/or tissue of an animal. Most non-viral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral gene delivery systems rely on endocytic pathways for the uptake of the novel HDAC polypeptide-encoding gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In clinical settings, the gene delivery systems for a therapeutic HDAC gene can be introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systematically, e.g., by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from the specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof.

In other aspects. the initial delivery of a recombinant HDAC gene is more limited, for example, with introduction into an animal being quite localized. For instance, the gene delivery vehicle can be introduced by catheter (see, U.S. Patent No. 5,328,470) or by stereotactic injection (e.g., Chen et al., 1994, *Proc. Natl. Acad. Sci. USA*, 91:3054-3057). An HDAC nucleic acid sequence (gene), e.g., sequences represented by SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and/or SEQ ID NO:96, or a fragment thereof, can be delivered in a gene therapy construct by electroporation using techniques described, for example, by Dev et al. (1994, *Cancer Treat. Rev.*, 20:105-115).

The gene encoding an HDAC polypeptide can be turned off by transforming a cell or tissue with an expression vector that expresses high levels of an HDAC polypeptide-encoding polynucleotide, or a fragment thereof. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are

disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and even longer if appropriate replication elements are designed to be part of the vector system.

Modifications of gene expression can be obtained by designing antisense molecules or complementary nucleic acid sequences (DNA, RNA, or PNA), to the control, 5', or regulatory regions of the genes encoding the novel HDAC polypeptides, (e.g., signal sequence, promoters, enhancers, and introns). Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferable. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described (See, for example, J.E. Gee et al., 1994, In: B.E. Huber and B.I. Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, NY). The antisense molecule or complementary sequence may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, i.e., enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Suitable examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding the HDAC polypeptides.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be

evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes according to the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. Such methods include techniques for chemically synthesizing oligonucleotides, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the human HDACs of the present invention. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP. Alternatively, the cDNA constructs that constitutively or inducibly synthesize complementary HDAC RNA can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl (rather than phosphodiesterase linkages) within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and are equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods that are well known in the art.

In another embodiment of the present invention, an expression vector containing the complement of the polynucleotide encoding an HDAC polypeptide, or an antisense HDAC oligonucleotide, may be administered to

an individual to treat or prevent a disease or disorder associated with uncontrolled or neoplastic cell growth, hyperactivity or stimulation, for example. A variety of specialized oligonucleotide delivery techniques may be employed, for example, encapsulation in unilamellar liposomes and reconstituted Sendai virus envelopes for RNA and DNA delivery (Arad et al., 1986, *Biochem. Biophys. Acta.*, 859:88-94).

In another embodiment, the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the present invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above may be applied to any individual in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Another aspect of the present invention involves a method for modulating one or more of growth, differentiation, or survival of a mammalian cell by modulating HDAC bioactivity, e.g., by inhibiting the deacetylase activity of HDAC proteins, or disrupting certain protein-protein interactions. In general, whether carried out *in vivo*, *in vitro*, *ex vivo*, or *in situ*, the method comprises treating a cell with an effective amount of an HDAC therapeutic so as to alter, relative to an effect in the absence of treatment, one or more of (i) rate of growth or proliferation, (ii) differentiation, or (iii) survival of the cell. Accordingly, the method can be carried out with HDAC therapeutics, such as peptide and peptidomimetics, or other molecules identified in the drug screening methods as described herein which antagonize the effects of a naturally-occurring HDAC protein on a cell.

Other HDAC therapeutics include antisense constructs for inhibiting expression of HDAC proteins, and dominant negative mutants of HDAC proteins which competitively inhibit protein-substrate and/or protein- protein interactions upstream and downstream of the wild-type HDAC protein. In an exemplary embodiment, an antisense method is used to treat tumor cells by antagonizing HDAC activity and blocking cell cycle progression. The method includes, but is not limited to, the treatment of testicular cells, so as modulate spermatogenesis; the modulation of osteogenesis or chondrogenesis, comprising the treatment of osteogenic cells or chondrogenic cell, respectively, with an HDAC polypeptide. In addition, HDAC polypeptides can be used to modulate the differentiation of progenitor cells, e.g., the method can be used to cause differentiation of hematopoietic cells, neuronal cells, or other stem/progenitor cell populations, to maintain a cell in a differentiated state, and/or to enhance the survival of a differentiated cell, e.g., to prevent apoptosis or other forms of cell death.

The present method is applicable, for example, to cell culture techniques, such as in the culturing of hematopoietic cells and other cells whose survival or differentiation state is dependent on HDAC function. Moreover, HDAC agonists and antagonists can be used for therapeutic intervention, such as to enhance survival and maintenance of cells, as well as to influence organogenic pathways, such as tissue patterning and other differentiation processes. As an example, such a method is practiced for modulating, in an animal, cell growth, cell differentiation or cell survival, and comprises administering a therapeutically effective amount of an HDAC polypeptide to alter, relative the absence of HDAC treatment, one or more of (i) rate of cell growth or proliferation, (ii) cell differentiation, and/or (iii) cell survival of one or more cell types in an animal.

In another of its aspects the present invention provides a method of determining if a subject, e.g., a human patient, is at risk for a disorder characterized by unwanted cell proliferation or aberrant control of differentiation. The method includes detecting, in a tissue of the subject, the presence or the absence of a genetic lesion characterized by at least one of

(i) a mutation of a gene encoding an HDAC protein, e.g. represented in one of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, or a homolog thereof, or (ii) the mis-expression of an HDAC gene. More specifically, detecting the genetic lesion includes ascertaining the existence of at least one of a deletion of one or more nucleotides from an HDAC gene; an addition of one or more nucleotides to the gene, a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene; an alteration in the level of a messenger RNA transcript of the gene; the presence of a non-wild type splicing pattern of an mRNA transcript of the gene; or a non-wild type level of the protein.

For example, detecting a genetic lesion can include (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence of an HDAC gene, e.g., a nucleic acid represented in one of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, or naturally occurring mutants thereof, or 5' or 3' flanking sequences naturally associated with the HDAC gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and (iii) detecting, by hybridization of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion; e.g., wherein detecting the lesion comprises utilizing the probe/primer to determine the nucleotide sequence of the HDAC gene and, optionally, of the flanking nucleic acid sequences. For instance, the probe/primer can be employed in a polymerase chain reaction (PCR) or in a ligation chain reaction (LCR). In alternative embodiments, the level of an HDAC protein is detected in an immunoassay using an antibody that is specifically immunoreactive with the HDAC protein.

Methods And Therapeutic Uses Related To Cell Modulation

Another aspect of the present invention relates to a method of inducing and/or maintaining a differentiated state, enhancing survival, and/or inhibiting (or alternatively, potentiating) the proliferation of a cell, by contacting cells with an agent that modulates HDAC-dependent transcription. In view of the apparently broad involvement of HDAC proteins in the control of chromatin

structure and, in turn, transcription and replication, the present invention contemplates a method for generating and/or maintaining an array of different tissue both *in vitro* and *in vivo*. An "HDAC therapeutic," whether inhibitory or potentiating with respect to modulating histone deacetylation, can be, as appropriate, any of the preparations described herein, including isolated polypeptides, gene therapy constructs, antisense molecules, peptidomimetics, or agents identified in the drug and bioactive screening assays and methods described herein.

As an aspect of the present invention, the HDAC modulatory (i.e., inhibitory or stimulatory) compounds are likely to play an important role in effecting cellular proliferation. There are a wide variety of pathological cell proliferative conditions for which HDAC therapeutic agents of the present invention may be used in treatment. For instance, such agents can provide therapeutic benefits in the inhibition of an anomalous cell proliferation. Nonlimiting examples of diseases and conditions that may benefit from such methods include various cancers and leukemias, psoriasis, bone diseases, fibroproliferative disorders, e.g., those involving connective tissues, atherosclerosis and other smooth muscle proliferative disorders, as well as chronic inflammation.

Non-limiting cancer types include carcinoma (e.g., adenocarcinoma), sarcoma, myeloma, leukemia, and lymphoma, and mixed types of cancers, such as adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, and teratocarcinoma. Representative cancers include, but are not limited to, bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer, prostate cancer, and melanoma. Specifically included are AIDS-related cancers (e.g., Kaposi's Sarcoma, AIDS-related lymphoma), bone cancers (e.g., osteosarcoma, malignant fibrous histiocytoma of bone, Ewing's Sarcoma, and related cancers), and hematologic/blood cancers (e.g., adult acute lymphoblastic leukemia, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia,

cutaneous T-cell lymphoma, adult Hodgkin's disease, childhood Hodgkin's disease, Hodgkin's disease during pregnancy, mycosis fungoides, adult non-Hodgkin's lymphoma, childhood non-Hodgkin's lymphoma, non-Hodgkin's lymphoma during pregnancy, primary central nervous system lymphoma, Sezary syndrome, cutaneous T-cell lymphoma, Waldenström's macroglobulinemia, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, and myeloproliferative disorders).

Also included are brain cancers (e.g., adult brain tumor, childhood brain stem glioma, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, childhood ependymoma, childhood medulloblastoma, supratentorial primitive neuroectodermal and pineal, and childhood visual pathway and hypothalamic glioma), digestive/gastrointestinal cancers (e.g., anal cancer, extrahepatic bile duct cancer, gastrointestinal carcinoid tumor, colon cancer, esophageal cancer, gallbladder cancer, adult primary liver cancer, childhood liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, and gastric cancer), musculoskeletal cancers (e.g., childhood rhabdomyosarcoma, adult soft tissue sarcoma, childhood soft tissue sarcoma, and uterine sarcoma), and endocrine cancers (e.g., adrenocortical carcinoma, gastrointestinal carcinoid tumor, islet cell carcinoma (endocrine pancreas), parathyroid cancer, pheochromocytoma, pituitary tumor, and thyroid cancer).

Further included are neurologic cancers (e.g., neuroblastoma, pituitary tumor, and primary central nervous system lymphoma), eye cancers (e.g., intraocular melanoma and retinoblastoma), genitourinary cancers (e.g., bladder cancer, kidney (renal cell) cancer, penile cancer, transitional cell renal pelvis and ureter cancer, testicular cancer, urethral cancer, Wilms' tumor and other childhood kidney tumors), respiratory/thoracic cancers (e.g., non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, and malignant thymoma), germ cell cancers (e.g., childhood extracranial germ cell tumor and extragonadal germ cell tumor), skin cancers (e.g., melanoma, and merkel cell carcinoma), gynecologic cancers (e.g., cervical cancer, endometrial cancer, gestational trophoblastic tumor, ovarian epithelial cancer,

ovarian germ cell tumor, ovarian low malignant potential tumor, uterine sarcoma, vaginal cancer, and vulvar cancer), and unknown primary cancers.

In certain aspects of the inventions, the disclosed HDAC inhibitors, antisense molecules, anti-HDAC antibodies, or antibody fragments can be used as treatments for breast or prostate cancers. In particular, HDAC9c inhibitors, HDAC9c antisense molecules, anti-HDAC9c antibodies, or fragments thereof, can be used. Specific breast cancers include, but are not limited to, non-invasive cancers, such as ductal carcinoma *in situ* (DCIS), intraductal carcinoma lobular carcinoma *in situ* (LCIS), papillary carcinoma, and comedocarcinoma, or invasive cancers, such as adenocarcinomas, or carcinomas, e.g., infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous (colloid) carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma. Specific prostate cancers may include adenocarcinomas and sarcomas, or pre-cancerous conditions, such as prostate intraepithelial neoplasia (PIN).

In addition to proliferative disorders, the present invention envisions the use of HDAC therapeutics for the treatment of differentiation disorders resulting from, for example, de-differentiation of tissue which may (optionally) be accompanied by abortive reentry into mitosis, e.g. apoptosis. Such degenerative disorders include chronic neurodegenerative diseases of the nervous system, including Alzheimer's disease, Parkinson's disease, Huntington's chorea, amylotrophic lateral sclerosis (ALS) and the like, as well as spinocerebellar degenerations. Other differentiation disorders include, for example, disorders associated with connective tissue, such as can occur due to de-differentiation of chondrocytes or osteocytes, as well as vascular disorders which involve de-differentiation of endothelial tissue and smooth muscle cells, gastric ulcers characterized by degenerative changes in glandular cells, and renal conditions marked by failure to differentiate, e.g. Wilm's tumors.

It will also be recognized that, by transient use of modulators of HDAC activities, *in vivo* reformation of tissue can be accomplished, for example, in

the development and maintenance of organs. By controlling the proliferative and differentiation potential for different cell types, HDAC therapeutics can be used to re-form injured tissue, or to improve grafting and morphology of transplanted tissue. As an example, HDAC antagonists and agonists can be employed in a differential manner to regulate different stages of organ repair after physical, chemical or pathological insult or injury. Such regimens can be utilized, for example, in the repair of cartilage, increasing bone density, liver repair subsequent to a partial hepatectomy, or to promote regeneration of lung tissue in the treatment of emphysema.

The present method is also applicable to cell culture techniques.

More specifically, HDAC therapeutics can be used to induce differentiation of uncommitted progenitor cells, thus giving rise to a committed progenitor cell, or causing further restriction of the developmental fate of a committed progenitor cell toward becoming a terminally differentiated cell. As an example, methods involving HDAC therapeutics can be used *in vitro*, *ex vivo*, or *in vivo* to induce and/or to maintain the differentiation of hematopoietic cells into erythrocytes and other cells of the hematopoietic cell lineage. Illustratively, the effect of erythropoietin (EPO) on the growth of EPO-responsive erythroid precursor cells is increased to influence their differentiation into red blood cells. Also, as an example, the amount of EPO, or other differentiating agent, that is required for growth and/or differentiation is reduced based on the administration of an inhibitor of histone deacetylation. (PCT/US92/07737).

Accordingly, HDAC therapeutics as described, particularly those that antagonize HDAC deacetylase activity, can be administered alone or in conjunction with EPO, for example, in a suitable carrier, to vertebrates to promote erythropoiesis. Alternatively, *ex vivo* cell treatments are suitable. Similar types of treatments can be used for a variety of disease states, including use in individuals who require bone marrow transplants (e.g., patients with aplastic anemia, acute leukemias, recurrent lymphomas, or solid tumors). As an example, prior to receiving a bone marrow transplant, a recipient is prepared by ablating or removing endogenous hematopoietic stem

cells. Such treatment is typically performed by total body irradiation, or by delivery of a high dose of an alkylating agent or other chemotherapeutic cytotoxic agent (Anklesaria et al., 1987, *Proc. Natl. Acad. Sci. USA*), 84:7681-7685). Following the preparation of the recipient, donor bone marrow cells are injected intravenously. Optionally, HDAC therapeutics could be contacted with the cells *ex vivo* or administered to the subject with the re-implanted cells.

In addition, there may be cell-type specific HDAC proteins, and/or some cell types may be more sensitive to the modulation of HDAC deacetylase activities. Even within a cell type, the stage of differentiation or position in the cell cycle could influence a cell's response to a modulatory HDAC therapeutic agent. Accordingly, the present invention contemplates the use of agents that modulate histone deacetylase activity to specifically inhibit or activate certain cell types. As an illustrative example, T cell proliferation could be preferentially inhibited so as to induce tolerance by a procedure similar to that used to induce tolerance using sodium butyrate (see, for example, PCT/US93/03045). Accordingly, HDAC therapeutics may be used to induce antigen specific tolerance in any situation in which it is desirable to induce tolerance, such as autoimmune diseases, in allogeneic or xenogeneic transplant recipients, or in graft versus host (GVH) reactions. Tolerance is typically induced by presenting the tolerizing compound (e.g., an HDAC inhibitor compound) substantially concurrently with the antigen, i.e., within a time period that is reasonably close to that in which the antigen is administered. Preferably, the HDAC therapeutic is administered after presentation of the antigen, so that the cumulative effect will occur after the particular repertoire of $T_H$ cells begins to undergo clonal expansion. Additionally, the present invention contemplates the application of HDAC therapeutics for modulating morphogenic signals involved in organogenic pathways. Thus, it is apparent that compositions comprising HDAC therapeutics can be employed for both cell culture and therapeutic methods involving the generation and maintenance of tissue.

In a further aspect, HDAC therapeutics are useful in increasing the amount of protein produced by a cell, including a recombinant cell. Suitable cells may comprise any primary cell isolated from any animal, cultured cells, immortalized cells, transfected or transformed cells, and established cell lines. Animal cells preferably will include cells which intrinsically have an ability to produce a desired protein; cells which are induced to have an ability to produce a desired protein, for example, by stimulation with a cytokine such as an interferon or an interleukin; genetically engineered cells into which a gene encoding a desired protein is introduced. The protein produced by the process can include peptides or proteins, including peptide-hormone or proteinaceous hormones such as any useful hormone, cytokine, interleukin, or protein which it may be desirable to be produced in purified form and/or in large quantity.

In specific aspects, the HDAC inhibitors, antisense molecules, anti-HDAC antibodies, or antibody fragments of the invention can be used in combination with other HDAC inhibitory agents, e.g., trichostatin A (D.M. Vigushin et al., 2001, *Clin. Cancer Res.* 7(4):971-6); trapoxin A (Itazaki et al., 1990, *J. Antibiot.* 43:1524-1532), MS-275 (T. Suzuki et al., 1999, *J. Med. Chem.* 42(15):3001-3), CHAPs (Y. Komatsu et al., 2001, *Cancer Res.* 61(11):4459-66), CI-994 (see, e.g., P.M. LoRusso et al., 1996, *New Drugs* 14(4):349-56), SAHA (V.M. Richon et al., 2001, *Blood Cells Mol. Dis.* 27(1):260-4), depsipeptide (FR901228; FK228; V. Sandor et al., 2002, *Clin. Cancer Res.* 8(3):718-28), CBHA (D.C. Coffey et al., 2001, *Cancer Res.* 61(9):3591-4), pyroxamide, (L.M. Butler et al, 2001, *Clin. Cancer Res.* 7(4):962-70), CHAP31 (Y. Komatsu et al., 2001, *Cancer Res.* 61(11):4459-66), HC-toxin (Liesch et al., 1982, *Tetrahedron* 38:45-48), chlamydocin (Closse et al., 1974, *Helv. Chim. Acta* 57:533-545), Cly-2 (Hirota et al., 1973, *Agri. Biol. Chem.* 37:955-56), WF-3161 (Umehana et al., 1983, *J. Antibiot.* 36, 478-483; M. Kawai et al., 1986, *J. Med. Chem.* 29(11):2409-11), Tan-1746 (Japanese Patent No. 7196686 to Takeda Yakuhin Kogyo KK), apicidin (S.H. Kwon et al., 2002, *J. Biol. Chem.* 277(3):2073-80), and analogs thereof.

Screening Methods

The novel HDAC proteins, peptides and nucleic acids can be used in screening assays to identify candidate bioactive agents or drugs that modulate HDAC bioactivity, preferably HDAC inhibitors, for potential use to treat neoplastic disorders, for example, to kill cancer cells and tumor cells exhibiting uncontrolled cell growth for numerous reasons, e.g., the lack of a suppressor molecule such as p53. In addition, HDAC proteins and encoding nucleic acids, as well as the bioactive agents that modulate HDAC activity or function, can be used as effectors in methods to regulate cell growth, e.g., to kill neoplastic cells.

The HDAC polynucleotides and polypeptides can also be modulated by interactive molecules. By "modulate" herein is meant that the bioactivity of HDAC is altered, i.e., either increased or decreased. In a preferred embodiment, HDAC function is inhibited. The HDACs can be used as targets to screen for inhibitors of HDAC, e.g., naturally-occurring HDAC, function, bioactivity, or expression in neoplastic cells and/or uncontrolled cell growth. Examples of HDAC biological activity include the ability to modulate the proliferation of cells. For example, inhibiting histone deacetylation causes cells to arrest in the G1 and G2 phases of the cell cycle. The biochemical activity associated with the novel HDAC proteins of the present invention are also characterized in terms of binding to and (optionally) catalyzing the deacetylation of an acetylated histone. Another biochemical property of certain HDAC proteins involves binding to other cellular proteins, such as RbAp48 (Qian et al., 1993, *Nature*, 364:648), or Sin3A. (see, e.g., WO 97/35990)

Generally, in performing screening methods, HDAC polypeptide or peptide can be non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g. a microtiter plate, an array, etc.). The criteria for suitable insoluble supports are that they can be made of any composition to which polypeptides can be bound; they are readily separated from soluble material; and they are otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any

convenient size or shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose. Microtiter plates and arrays are especially convenient, because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding the polypeptide is not crucial, so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the peptide and is nondiffusable.

Preferred methods of binding include the use of antibodies (which should not hinder the binding of HDACs to associated proteins), direct binding to "sticky" or ionic supports, chemical crosslinking, etc. Following binding of the polypeptide, excess unbound material is removed by washing. The sample receiving areas may then be blocked as needed through incubation with bovine serum albumin (BSA), casein or other innocuous/nonreactive protein.

A candidate bioactive agent is added to the assay. Novel binding agents include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The term "agent" as used herein describes any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., having the capability of directly or indirectly altering the activity or function of HDAC polypeptides. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration, or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 10,000

daltons, preferably, less than about 2000 to 5000 daltons, as a nonlimiting example. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. In addition, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

The determination of the binding of the candidate biomolecule or agent to an HDAC polypeptide may be accomplished in a number of ways practiced in the art. In one aspect, the candidate bioactive agent is labeled, and binding is determined directly. Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, enzymes, fluorescent and chemiluminescent compounds, specific binding molecules, particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which allows detection, in

accordance with known procedures. In some embodiments, only one of the components is labeled. Alternatively, more than one component may be labeled with different labels; for example, the HDAC polypeptide may be labeled with one fluorophor and the candidate agent labeled with another

In one embodiment, the candidate bioactive agent is labeled. Labeled candidate bioactive agents are incubated with an HDAC polypeptide for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4ºC and 40ºC. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high throughput screening. Typically between 0.1 and 1 hour is sufficient. Excess reagent is generally removed or washed away. The presence or absence of the labeled component is detected to determine and indicate binding.

A variety of other reagents may be included in the screening assay. Such reagents include, but are not limited to, salts, neutral proteins, e.g. albumin, detergents, etc., which may be used to facilitate optimal protein-protein binding and/or to reduce non-specific or background interactions. In addition, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. Further, the mixture of components in the method may be added in any order that provides for the requisite binding.

Kits are included as an embodiment of the present invention which comprise containers with reagents necessary to screen test compounds. Depending on the design of the test and the types of compounds to be screened, such kits include human HDAC polynucleotide, polypeptide, or peptide and instructions for performing the assay.

Inhibitors of the enzymatic activity of each of the novel HDAC polypeptides can be identified using assays which measure the ability of an agent to inhibit catalytic conversion of a substrate by the HDAC proteins provided by the present invention. For example, the ability of the novel HDAC proteins to deacetylate a histone substrate, such as histone H4, in the

presence and absence of a candidate inhibitor, can be determined using standard enzymatic assays.

A number of methods have been employed in the art for assaying histone deacetylase activity, and can be incorporated in the drug screening assays of the present invention. Preferably, the assay method will employ a labeled acetyl group linked to appropriate histone lysine residues as substrates. In other embodiments, a histone substrate peptide can be labeled with a group whose signal is dependent on the simultaneous presence or absence of an acetyl group, e.g., the label can be a fluorogenic group whose fluorescence is modulated (either quenched or potentiated) by the presence of the acetyl moiety.

Using standard enzymatic analysis, the ability of a test agent (i.e., test compound) to cause a statistically significant change in substrate conversion by a histone deacetylase can be measured, and as desirable, inhibition constants, e.g., $K_i$ values, can be calculated. The histone substrate can be provided as a purified or semi-purified polypeptide or as part of a cell lysate. Likewise, the histone deacetylase can be provided to a reaction mixture as a purified or semi-purified polypeptide, or as a cell lysate. Accordingly, the reaction mixtures can range from reconstituted protein mixtures derived with purified preparations of histones and deacetylases, to mixtures of cell lysates, e.g., by admixing baculovirus lysates containing recombinant histones and deacetylases.

As an example, the histone substrate for assays described herein can be provided by isolation of radiolabeled histones from metabolically labeled cells. Cells such as HeLa cells can be labeled in culture by the addition of [$^3$H]acetate (New England Nuclear) to the culture media. (Hay et al., 1983, *J. Biol. Chem.*, 258:3726-3734). The addition of an HDAC inhibitor, such as butyrate, trapoxin and the like, can be used to increase the abundance of acetylated histones in the cells. Radiolabeled histones can be isolated from the cells by extraction with $H_2S0_4$ (Marushige et al., 1966, *J. Mol. Biol.*, 15:160-174). Briefly, cells are homogenized in buffer, centrifuged to isolate a nuclear pellet, and the subsequently homogenized nuclear pellet is

centrifuged through sucrose. The resulting chromatin pellet extracted by addition of $H_2SO_4$ to yield [$^3$H]acetyl-labeled histones. Alternatively, nucleosome preparations containing [$^3$H]acetyl-labeled histones can be isolated from metabolically labeled cells. As known in the art, nucleosomes can be isolated from cell preparations by sucrose gradient centrifugation (e.g., Hay et al., 1983, *J. Biol. Chem.*, 258:3726-3734 and Noll, 1967, *Nature*, 215:360-363), and polynucleosomes can be prepared by NaCl precipitation from micrococcal nuclease digested cells (Hay et al., *supra*).

Similar procedures for isolating labeled histones from other cells types, including yeast, have been described. (See for example, Alonso et al., 1986, *Biochem Biophys Acta*, 866:161-169 and Kreiger et al, 1974, *J. Biol. Chem.*, 249:332 334). Also, histones are generated by recombinant gene expression, and include an exogenous tag (e.g., an HA epitope, a poly(his) sequence, and the like) which facilitates purification from cell extracts. Further, whole nuclei can be isolated from metabolically labeled cells by micrococcal nuclease digestion (Hay et al., *supra*).

The deacetylase substrate can also be provided as an acetylated peptide including a sequence corresponding to the sequence around the specific lysyl residues acetylated on histones, e.g., peptidyl portions of the core histones H2A, H2B, H3, or H4. Such fragments can be produced by cleavage of acetylated histones derived from metabolically labeled cells, e.g., by treatment with proteolytic enzymes or cyanogen bromide (Kreiger et al., *supra*). The acetylated peptide can also be provided by standard solid phase synthesis using acetylated lysine residues (*Id.*).

The activity of a histone deacetylase in assay detection methods involving use of [$^3$H]acetyl-labeled histones is detected by measuring the release of [$^3$H]acetate by standard scintillation techniques. As an illustrative example, a reaction mixture is provided which contains a recombinant HDAC protein suspended in buffer, along with a sample of [$^3$H]acetyl-labeled histones and (optionally) a test compound. The reaction mixture is maintained at a desired temperature and pK such as 22°C at pH 7.8, for several hours, and the reaction is terminated by boiling, or another form of

denaturation. Released [$^3$H]acetate is extracted and counted. For example, the quenched reaction mixture can be acidified with concentrated HCl and used to create a biphasic mixture with ethyl acetate. The resulting two-phase system is thoroughly mixed, centrifuged, and the ethyl acetate phase collected and counted by standard scintillation methods. Other methods for detecting acetate release will be easily recognized by those having skill in the art.

In yet another aspect, the drug screening assay is designed to include a reagent cell recombinantly expressing one or more of a target protein or HDAC protein. The ability of a test agent to alter the activity of the HDAC protein can be detected by analysis of the recombinant cell. For instance, agonists and antagonists of the HDAC biological activity can by detected by scoring for alterations in growth or differentiation (phenotype) of the cell. General techniques for detecting these characteristics are well known, and will vary with respect to the source of the particular reagent cell utilized in any given assay. For example, quantification of cell proliferation in the presence and absence of a candidate agent can be measured by using a number of techniques well known in the art, including simple measurement of population growth curves.

Where an assay involves proliferation in a liquid medium, turbidimetric techniques (i.e. absorbance/transmittance of light of a given wavelength through the sample) can be utilized. For example, in a case in which the reagent cell is a yeast cell, measurement of absorbance of light at a wavelength at between 540 and 600 nm can provide a conveniently fast measure of cell growth. Moreover, the ability of yeast cells to form colonies in solid medium (e.g. agar) can be used to readily score for proliferation. In other embodiments, an HDAC substrate protein, such as a histone, can be provided as a fusion protein which permits the substrate to be isolated from cell lysates and the degree of acetylation detected. Each of these techniques is suitable for high throughput analysis necessary for rapid screening of large numbers of candidate HDAC modulatory agents.

In addition, in assays in which the ability of an agent to cause or reverse a transformed phenotype is being determined, cell growth in solid or semi-solid medium, such as agar, can further aid in establishing whether a mammalian cell is transformed. Visual inspection of the morphology of the reagent cell can also be used to determine whether the biological activity of the targeted HDAC protein has been affected by the added agent. By illustration, the ability of an agent to influence an apoptotic phenotype which is mediated in some way by a recombinant HDAC protein can be assessed by visual microscopy. Similarly, the formation of certain cellular structures as part of normal cell differentiation, such as the formation of neuritic processes, can be visualized under a light microscope.

The nature of the effect of a test agent on a reagent cell can be assessed by measuring levels of expression of specific genes, e.g., by reverse transcription PCR. Another method of scoring for an effect on HDAC activity is by detecting cell-type specific marker expression through immunofluorescent staining. Many such markers are known in the art for which antibodies are readily available. For example, the presence of chondroitin sulfate proteoglycans, as well as type-II collagen, is correlated with cartilage production in chondrocytes, and each can be detected by immunostaining. Similarly, the human kidney differentiation antigen gp160, human aminopeptidase A, is a marker of kidney induction, and the cytoskeletal protein troponin I is a marker of heart induction.

Also, the alteration of expression of a reporter gene construct provided in the reagent cell provides a means of detecting an effect on HDAC activity. For example, reporter gene constructs designed using transcriptional regulatory sequences, e.g. the promoters, for developmentally regulated genes can be used to drive the expression of a detectable marker, such as a luciferase gene. For example, the construct can be prepared using the promoter sequence from a gene expressed in a particular differentiation phenotype.

Pharmaceutical Compositions

A further embodiment of the present invention embraces the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, diluent, or excipient, for any of the above-described therapeutic uses and effects. Such pharmaceutical compositions may comprise HDAC nucleic acid, polypeptide, or peptides, antibodies to HDAC polypeptides or peptides, or fragments thereof, mimetics, agonists (e.g., activators), antagonists (e.g., inhibitors, blockers) of the HDAC polypeptide, peptide, or polynucleotide. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical (or physiologically compatible) carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, hormones, or biological response modifiers. Preferred are compositions comprising one or more HDAC inhibitors.

The pharmaceutical compositions for use in the present invention can be administered by any number of routes including, but not limited to, parenteral oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, ophthalmic, enteral, topical, sublingual, vaginal, or rectal means.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing a deacetylase inhibitor in the proper medium. Absorption enhancers can also be used to increase the flux of the deacetylase inhibitor across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the deacetylase inhibitor in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

In addition to the active ingredients (i.e., an HDAC antagonist compound), the pharmaceutical compositions may contain suitable

pharmaceutically acceptable carriers or excipients comprising auxiliaries which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Further details on techniques for formulation and administration are provided in the latest edition of *Remington's Pharmaceutical Sciences* (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained by the combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropyl-methylcellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth, and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a physiologically acceptable salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with physiologically suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification, or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, scaled capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain

active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. In addition, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyloleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants or permeation agents that are appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants and permeation enhancers are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, and the like. Salts tend to be more soluble in aqueous solvents, or other protonic solvents, than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to

5.5, combined with a buffer prior to use. After the pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of an HDAC inhibitor compound, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose or amount is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., using neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used and extrapolated to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example, an HDAC inhibitor or antagonist compound, antibodies to an HDAC polypeptide or peptide, agonists of HDAC polypeptides, which ameliorates, reduces, or eliminates the symptoms or the condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., $ED_{50}$ (the dose therapeutically effective in 50% of the population) and $LD_{50}$ (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio, $LD_{50}/ED_{50}$. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in determining a range of dosages for human use. Preferred dosage contained in a pharmaceutical composition is within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, who will consider the factors related to the individual requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the individual's disease state, general health of the patient, age, weight, and gender of the patient, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. As a general guide, long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks, depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms (μg), up to a total dose of about 1 gram (g), depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and is generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, and the like.

Assays and Diagnostics

In another embodiment of the present invention, antibodies which specifically bind to the HDAC polypeptides or peptides of the present invention may be used for the diagnosis of conditions or diseases characterized by expression (or overexpression) of an HDAC polynucleotide or polypeptide, or in assays to monitor patients being treated modulatory compounds of HDAC polypeptides, or, for example, HDAC antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for use in therapeutic methods. Diagnostic assays for the HDAC polypeptides include methods which utilize the antibody and a label to detect the protein in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a

reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

Several assay protocols including ELISA, RIA, and FACS for measuring an HDAC polypeptide or peptide are known in the art and provide a basis for diagnosing altered or abnormal levels of HDAC polypeptide expression. Normal or standard values for HDAC polypeptide expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HDAC polypeptide or peptide under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods; photometric means are preferred. Quantities of HDAC polypeptide or peptide expressed in subject sample, control sample, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In one embodiment of the present invention, anti-HDAC antibodies (e.g., anti-HDAC9c antibodies) can be used in accordance with established methods to detect the presence of specific cancers or tumors, such as breast or prostate cancers or tumors. Representative cancers and cancer types are listed above.

According to another embodiment of the present invention, the polynucleotides encoding the novel HDAC polypeptides may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify HDAC-encoding nucleic acid expression in biopsied tissues in which expression (or under- or overexpression) of HDAC polynucleotide may be correlated with disease. The diagnostic assay may be used to distinguish between the absence, presence, and excess expression of HDAC, and to monitor regulation of HDAC polynucleotide levels during therapeutic treatment or intervention.

In a related aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding an HDAC polypeptide, or closely related molecules, may be used to identify nucleic acid sequences which encode an HDAC polypeptide. The specificity of the probe, whether it is made from a highly specific region, e.g., about 8 to 10 or 12 or 15 contiguous nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding the HDAC polypeptide, alleles thereof, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50%, preferably at least 80%, of the nucleotides encoding an HDAC polypeptide. The hybridization probes of this invention may be DNA or RNA and may be derived from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring HDAC protein.

The nucleotide sequences of the novel HDAC genes presented herein will further allow for the generation of probes and primers designed for use in identifying and/or cloning HDAC homologs in other cell types, e.g. from other tissues, as well as HDAC homologs from other organisms. For example, the present invention also provides a probe/primer comprising a substantially purified oligonucleotide, which oligonucleotide comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least 10 consecutive nucleotides of sense or anti-sense sequence selected from the group consisting of HDAC SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, or naturally occurring mutants thereof. Primers based on the nucleic acid represented in SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, or as presented in the tables herein, can be used in PCR

reactions to clone HDAC homologs. Likewise, probes based on the HDAC sequences provided herein can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. The probe preferably comprises a label moiety attached thereto and is able to be detected, e.g., the label moiety is selected from radioisotopes, fluorescent compounds, chemiluminescent compounds, enzymes, enzyme co-factors, and the like.

Such probes can also be used as a part of a diagnostic test kit for identifying cells or tissue which mis-express an HDAC protein, such as by measuring a level of an HDAC encoding nucleic acid in a sample of cells from a patient; e.g., detecting HDAC mRNA levels, or determining whether a genomic HDAC gene has been mutated or deleted. To this end, nucleotide probes can be generated from the HDAC sequences herein which facilitate histological screening of intact tissue and tissue samples for the presence (or absence) of HDAC-encoding transcripts. Similar to the diagnostic uses of anti-HDAC antibodies, the use of probes directed to HDAC messages, or to genomic HDAC sequences, can be used for both predictive and therapeutic evaluation of allelic mutations which might be manifest in, for example, neoplastic or hyperplastic disorders (e.g. unwanted cell growth), or the abnormal differentiation of tissue. Used in conjunction with immunoassays as described herein, the oligonucleotide probes can help facilitate the determination of the molecular basis for a developmental disorder which may involve some abnormality associated with expression (or lack thereof) of an HDAC protein. For instance, variation in polypeptide synthesis can be differentiated from a mutation in a coding sequence.

Accordingly, the present invention provides a method for determining if a subject is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. Such a method can be generally characterized as comprising detecting, in a sample of cells from a subject, the presence or absence of a genetic lesion characterized by at least one of (i) an alteration affecting the integrity of a gene or nucleic acid sequence encoding an HDAC polypeptide, or (ii) the mis-expression of an HDAC gene. To illustrate, such genetic lesions can be detected by ascertaining the existence of at least one

of (i) a deletion of one or more nucleotides from an HDAC gene, (ii) an addition of one or more nucleotides to an HDAC gene, (iii) a substitution of one or more nucleotides of an HDAC gene, (iv) a gross chromosomal rearrangement of an HDAC gene, (v) a gross alteration in the level of a messenger RNA transcript of an HDAC gene, (vii) aberrant modification of an HDAC gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an HDAC gene, (viii) a non-wild type level of an HDAC polypeptide, and (ix) inappropriate post-translational modification of an HDAC polypeptide. Accordingly, the present invention provides a large number of assay techniques for detecting lesions in an HDAC gene, and importantly, provides the ability to distinguish between different molecular causes underlying HDAC-dependent aberrant cell growth, proliferation and/or differentiation.

Methods for producing specific hybridization probes for DNA encoding the HDAC polypeptides include the cloning of nucleic acid sequence that encodes the HDAC polypeptides, or HDAC derivatives, into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of detector/reporter groups, e.g., radionuclides such as $^{32}P$ or $^{35}S$, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/ biotin coupling systems, and the like.

The polynucleotide sequences encoding the HDAC polypeptides may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect the status of, e.g., levels or overexpression of HDAC, or to detect altered HDAC expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding the HDAC polypeptides may be useful in assays that detect activation or induction of various tumors, neoplasms or cancers. The nucleotide sequences encoding

the HDAC polypeptides may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable control sample, the nucleotide sequence has hybridized with nucleotide sequence present in the sample, and the presence of altered levels of nucleotide sequence encoding the HDAC polypeptides in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In one embodiment of the present invention, HDAC (e.g., HDAC9c) nucleic acids can be used in accordance with established methods to detect the presence of specific cancers or tumors, such as breast or prostate cancers or tumors. Representative cancers and cancer types are listed herein above.

To provide a basis for the diagnosis of disease associated with HDAC expression, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes an HDAC polypeptide, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject (patient) values is used to establish the presence of disease.

Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is

observed in a normal individual. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier, thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the nucleic acid sequences encoding the novel HDAC polypeptides may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably comprise two nucleotide sequences, one with sense orientation (5'→3') and another with antisense (3'→5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

Methods suitable for quantifying the expression of HDAC include radiolabeling or biotinylating nucleotides, co-amplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (P.C. Melby et al., 1993, *J. Immunol. Methods*, 159:235-244; and C. Duplaa et al., 1993, *Anal. Biochem.*, 229-236). The speed of quantifying multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantification.

In another embodiment of the present invention, oligonucleotides, or longer fragments derived from the HDAC polynucleotide sequences described herein, may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to

produce a transcript image), and to identify genetic variants, mutations and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disease, to diagnose disease, and to develop and monitor the activities of therapeutic agents. In a particular aspect, the microarray is prepared and used according to the methods described in WO 95/11995 (Chee et al.); D.J. Lockhart et al., 1996, *Nature Biotechnology*, 14:1675-1680; and M. Schena et al., 1996, *Proc. Natl. Acad. Sci. USA*, 93:10614-10619). Microarrays are further described in U.S. Patent No. 6,015,702 to P. Lal et al.

In another embodiment of this invention, a nucleic acid sequence which encodes one or more of the novel HDAC polypeptides may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial PI constructions, or single chromosome cDNA libraries, as reviewed by C.M. Price, 1993, *Blood Rev.*, 7:127-134 and by B.J. Trask, 1991, *Trends Genet.*, 7:149-154.

In another embodiment of the present invention, an HDAC polypeptide, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between an HDAC polypeptide, or portion thereof, and the agent being tested, may be measured utilizing techniques commonly practiced in the art and as described above.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in WO 84/03564. In this method, as applied to HDAC protein, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with an HDAC polypeptide, or fragments

thereof, and washed. Bound HDAC polypeptide is then detected by methods well known in the art. Purified HDAC polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Other screening and small molecule (e.g., drug) detection assays which involve the detection or identification of small molecules that can bind to a given protein, i.e., an HDAC protein, are encompassed by the present invention. Particularly preferred are assays suitable for high throughput screening methodologies. In such binding-based screening or detection assays, a functional assay is not typically required. All that is needed is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small molecules) to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein will modulate activity in some manner, due to preferential, higher affinity binding to functional areas or sites on the protein.

An example of such an assay is the fluorescence based thermal shift assay (3-Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng. News* 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, HDAC polypeptide based on affinity of binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods, such as those described herein, to determine if the molecules affect or modulate function or activity of the target protein.

In a further embodiment of this invention, competitive drug screening assays can be used in which neutralizing antibodies capable of binding an HDAC polypeptide specifically compete with a test compound for binding to HDAC polypeptide. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with an HDAC polypeptide.

In yet another of its aspects, the present invention provides the identification of compounds with optimum therapeutic indices, or drugs or compounds which have therapeutic indices more favorable than known HDAC inhibitors, such as trapoxin, tichostatin, sodium butyrate, and the like. The identification of such compounds can be made by the use of differential screening assays which detect and compare drug mediated inhibition of deacetylase activity between two or more different HDAC-like enzymes, or which compare drug mediated inhibition of formation of complexes involving two or more different types of HDAC-like proteins.

For example, an assay can be designed for side-by side comparison of the effect of a test compound on the deacetylase activity or protein interactions of tissue-type specific HDAC proteins. Given the apparent diversity of HDAC proteins, it is probable that different functional HDAC activities, or HDAC complexes, exist and in certain instances, are localized to particular tissue or cell types. Thus, test compounds can be screened to identify agents that are able to inhibit the tissue-specific formation of only a subset of the possible repertoire of HDAC/regulatory protein complexes, or which preferentially inhibit certain HDAC enzymes. For instance, an "interaction trap assay" can be derived using two or more different human HDAC "bait" proteins, while the "fish" protein is constant in each, e.g., a human RbAp48 construct. Running the interaction trap side- by-side permits the detection of agents which have a greater effect (e.g., statistically significant) on the formation of one of the HDAC/RbAp48 complexes than on the formation of the other HDAC complexes. (See, e.g., WO 97/35990).

Similarly, differential screening assays can be used to exploit the difference in protein interactions and/or catalytic mechanisms of mammalian HDAC proteins and yeast RPD3 proteins, for example, in order to identify agents which display a statistically significant increase in specificity for inhibiting the yeast enzyme relative to the mammalian enzyme. Thus, lead compounds which act specifically on pathogens, such as fungus involved in mycotic infections, can be developed. By way of illustration, assays can be used to screen for agents which may ultimately be useful for inhibiting at least

one fungus implicated in pathologies such as candidiasis, aspergillosis, mucomycosis, blastomycosis, geotrichosis, cryptococcosis, chromoblastomycosis, coccidiomycosis, conidiosporosis, histoplasmosis, maduromycosis, rhinosporidosis, nocaidiosis, para actinomycosis, penicilliosis, monoliasis, or sporotrichosis.

As an example, if the mycotic infection to which treatment is desired is candidiasis, the described assay can involve comparing the relative effectiveness of a test compound on inhibiting the deacetylase activity of a mammalian HDAC protein with its effectiveness in inhibiting the deacetylase activity of an RPD3 homolog that has been cloned from yeast selected from the group consisting of *Candida albicans, Candida stellatoidea, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida pseudotropicalis, Candida quillermondii,* or *Candida rugosa.* Such an assay can also be used to identify anti-fungal agents which may have therapeutic value in the treatment of aspergillosis by selectively targeting RPD3 homologs cloned from yeast such as *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans*, or *Aspergillus terreus.* Where the mycotic infection is muco-mycosis, the RPD3 deacetylase can be derived from yeast such as *Rhizopus arrhizus, Rhizopus oryzae, Absidja corymbiera, Absidia ramosa*, or *Mucor pusillus.*

Sources of other RPD3 activities for comparison with a mammalian HDAC activity include the pathogen *Pneumocystis carinii.*

In addition to such HDAC therapeutic uses, anti-fungal agents developed from such differential screening assays can be used, for example, as preservatives in foodstuff, feed supplement for promoting weight gain in livestock, or in disinfectant formulations for treatment of non-living matter, e.g., for decontaminating hospital equipment and rooms. In a similar fashion, side by side comparison of the inhibition of a mammalian HDAC protein and an insect HDAC-related protein, will permit selection of HDAC inhibitors which are capable of discriminating between the human/mammalian and insect enzymes. Accordingly, the present invention envisions the use and

formulations of HDAC therapeutics in insecticides, such as for use in management of insects like the fruit fly.

In yet another embodiment, certain of the subject HDAC inhibitors can be selected on the basis of inhibitory specificity for plant HDAC-related activities relative to the mammalian enzyme. For example, a plant HDAC-related protein can be disposed in a differential screen with one or more of the human enzymes to select those compounds of greatest selectivity for inhibiting the plant enzyme. Thus, the present invention specifically contemplates formulations of HDAC inhibitors for agricultural applications, such as in the form of a defoliant or the like.

In many drug screening programs that test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be rapidly generated to permit the quick development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. In addition, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in an *in vitro* system, since the assay is focused primarily on the effect of the drug on the molecular target which may be manifest in an alteration of binding affinity with upstream or downstream elements.

Accordingly, in an exemplary screening assay, a reaction mixture is generated to include an HDAC polypeptide, compound(s) of interest, and a "target polypeptide", e.g., a protein, which interacts with the HDAC polypeptide, whether as a substrate or by some other protein-protein interaction. Exemplary target polypeptides include histones, RbAp48 polypeptides, p53 polypeptides, and/or combinations thereof, or with other transcriptional regulatory proteins (such as myc, max, etc.). Detection and quantification of complexes containing the HDAC protein provide a means for determining a compound's efficacy at inhibiting (or potentiating) complex formation between the HDAC and the target polypeptide. The efficacy of the

compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. In the control assay, isolated and purified HDAC polypeptide is added to a composition containing the target polypeptide and the formation of a complex is quantified in the absence of the test compound.

Complex formation between an HDAC polypeptide and the target polypeptide may be detected by a variety of techniques. Modulation of the formation of complexes can be quantified using, for example, detectably labeled proteins such as radiolabeled, fluorescently labeled, or enzymatically labeled HDAC polypeptides, by immunoassay, by chromatography, or by detecting the intrinsic activity of the acetylase.

Transgenics and Knock Outs

The present invention further encompasses transgenic non-human mammals, preferably mice, that comprise a recombinant expression vector harboring a nucleic acid sequence that encodes a human HDAC (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, or SEQ ID NO:95).

Transgenic non-human mammals useful to produce recombinant proteins are well known to the skilled practitioner, as are the expression vectors necessary and the techniques for generating transgenic animals. Generally, the transgenic animal comprises a recombinant expression vector in which the nucleotide sequence that encodes a human HDAC is operably linked to a tissue specific promoter whereby the coding sequence is only expressed in that specific tissue. For example, the tissue specific promoter can be a mammary cell specific promoter and the recombinant protein so expressed is recovered from the animal's milk.

The transgenic animals, particularly transgenic mice, containing a nucleic acid molecule which encodes a novel human HDAC may be used as animal models for studying *in vivo* the overexpression of HDAC and for use in drug evaluation and discovery efforts to find compounds effective to inhibit or modulate the activity of HDAC, such as for example compounds for treating

disorders, diseases, or conditions related to cell proliferation and neoplastic cell growth, for example. One having ordinary skill in the art using standard techniques, such as those taught in U.S. Patent No. 4,873,191, issued Oct. 10, 1989 to Wagner and in U.S. Patent No. 4,736,866, issued April 12, 1988 to Leder, can produce transgenic animals which produce human HDAC, and use the animals in drug evaluation and discovery projects.

The transgenic non-human animals according to this aspect of the present invention can express a heterologous HDAC-encoding gene, or which have had one or more genomic HDAC genes disrupted in at least one of the tissue or cell types of the animal. Accordingly, the invention features an animal model for developmental diseases, which animal has one or more HDAC alleles which are improperly expressed. For example, a mouse can be bred which has one or more HDAC alleles deleted or otherwise rendered inactive. Such a mouse model can then be used to study disorders arising from improperly expressed HDAC genes, as well as for evaluating potential therapies for similar disorders.

Another aspect of transgenic animals are those animals which contain cells harboring an HDAC transgene according to the present invention and which preferably express an exogenous HDAC protein in one or more cells in the animal. An HDAC transgene can encode the wild-type form of the protein, or can encode homologs thereof, including both agonists and antagonists, as well as antisense constructs. Preferably, the expression of the transgene is restricted to specific subsets of cells, tissues or developmental stages utilizing, for example, cis-acting sequences that control expression in the desired pattern. According to the invention, such mosaic expression of an HDAC protein can be essential for many forms of lineage analysis and can also provide a means to assess the effects of, for example, lack of HDAC expression which might grossly alter development in small portions of tissue within an otherwise normal embryo. Toward this end, tissue specific regulatory sequences and conditional regulatory sequences can be used to control the expression of the transgene in certain spatial patterns. Moreover,

temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory sequences.

Genetic techniques which allow for the expression of transgenes can be regulated via site-specific genetic manipulation *in vivo* are known to those skilled in the art. For instance, genetic systems are available which permit the regulated expression of a recombinase that catalyzes the genetic recombination of a target sequence. The phrase "target sequence" in this instance refers to a nucleotide sequence that is genetically recombined by a recombinase. The target sequence is flanked by recombinase recognition sequences and is generally either excised or inverted in cells expressing recombinase activity. Recombinase catalyzed recombination events can be designed such that recombination of the target sequence results in either the activation or repression of expression of one of the present HDAC proteins.

For example, excision of a target sequence which interferes with the expression of a recombinant HDAC gene, such as one which encodes an antagonistic homolog or an antisense transcript, can be designed to activate the expression of that gene. This interference with expression of an encoded product can result from a variety of mechanisms, such as spatial separation of the HDAC gene from the promoter element, or an internal stop codon. Moreover, the transgene can be made so that the coding sequence of the gene is flanked by recombinase recognition sequences and is initially transfected into cells in a 3' to 5' orientation with respect to the promoter element. In this case, inversion of the target sequence will reorient the subject gene by placing the 5' end of the coding sequence in an orientation with respect to the promoter element which allows for promoter driven transcriptional activation.

Illustratively, transgenic non-human animals are produced by introducing transgenes into the germline of the non-human animal. Embryonic target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonic target cell. The zygote is a preferred target for micro-injection.

In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (e.g., Brinster et al., 1985, *Proc. Natl. Acad. Sci. USA*, 82:4438-4442). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will generally also be reflected in the efficient transmission of the transgene to offspring of the founder mice since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating HDAC transgenes.

In addition, retroviral infection can also be used to introduce HDAC transgenes into a non human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres are targets for retroviral infection (R. Jaenisch, 1976, *Proc. Natl. Acad. Sci. USA.*, 73:1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Manipulating the Mouse Embryo, Hogan eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1986). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner et al., 1985, *Proc. Natl. Acad. Sci. USA.*, 82:6927 6931; Van der Putten et al., 1985, *Proc. Natl. Acad. Sci. USA.*, 82:6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Stewart et al., 1987, *EMBO J.*, 6:383-388).

Alternatively, infection can be performed at a later developmental stage. For example, virus or virus-producing cells can be injected into the blastocoele (e.g., Jahner et al., 1982, *Nature*, 298:623-628). Most of the founder animals win be mosaic for the transgene, because incorporation occurs only in the subset of cells which formed the transgenic non-human animal. Further, the founders may contain various retroviral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. It is also possible to introduce transgenes into the germline

by intrauterine retroviral infection of the midgestation embryo (Jahner et al., 1982, *supra*).

A third type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells are obtained from pre-implantation embryos that are cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature*, 292:154-156; Bradley et al., 1984, *Nature*, 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA.*, 83:9065-9069; and Robertson et al., 1986, *Nature*, 322:445-448). Cultured ES cell lines are available. Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. Transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells then colonize the embryo and contribute to the germ line of the resulting chimeric animal. See, e.g., R. Jaenisch, 1988, *Science*, 240:1468-1474.

Methods for making HDAC knock-out animals, or disruption transgenic animals are also generally known. See, for example, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Recombinase dependent knockouts can also be generated, e.g. by homologous recombination, to insert recombinase target sequences flanking portions of an endogenous HDAC gene, such that tissue specific and/or temporal control of inactivation of an HDAC gene sequence or allele can be controlled as above.

In knock-outs, transgenic mice may be generated which are homozygous for a mutated, non-functional HDAC gene which is introduced into the animals using well known techniques. Surviving knock-out mice produce no functional HDAC and thus are useful to study the function of HDAC. Furthermore, the mice may be used in assays to study the effects of test compounds in HDAC deficient animals. For instance, HDAC-deficient mice can be used to determine if, how and to what extent HDAC inhibitors will effect the animal and thus address concerns associated with inhibiting the activity of the molecule.

More specifically, methods of generating genetically deficient knock-out mice are well known and are disclosed in M.R. Capecchi, 1989, *Science*,

244:1288-1292 and P. Li et al., 1995, *Cell*, 80:401-411. For example, a human HDAC cDNA clone can be used to isolate a murine HDAC genomic clone. The genomic clone can be used to prepare an HDAC targeting construct which can disrupt the HDAC gene in the mouse by homologous recombination. The targeting construct contains a non-functioning portion of an HDAC gene which inserts in place of the functioning portion of the native mouse gene. The non-functioning insert generally contains an insertion in the exon that encodes the active region of the HDAC polypeptide. The targeting construct can contain markers for both positive and negative selection. The positive selection marker allows for the selective elimination of cells which do not carry the marker, while the negative selection marker allows for the elimination of cells that carry the marker.

For example, a first selectable marker is a positive marker that will allow for the survival of cells carrying it. In some instances, the first selectable marker is an antibiotic resistance gene, such as the neomycin resistance gene, which can be placed within the coding sequence of a novel HDAC gene to render it non-functional, while at the same time rendering the construct selectable. The antibiotic resistance gene is within the homologous region which can recombine with native sequences. Thus, upon homologous recombination, the non-functional and antibiotic resistance selectable gene sequences will be taken up. Knock-out mice may be used as models for studying inflammation-related disorders and screening compounds for treating these disorders.

The targeting construct also contains a second selectable marker which is a negative selectable marker. Cells with the negative selectable marker will be eliminated. The second selectable marker is outside the recombination region. Thus, if the entire construct is present in the cell, both markers will be present. If the construct has recombined with native sequences, the first selectable marker will be incorporated into the genome and the second will be lost. The herpes simplex virus thymidine kinase (HSV tk) gene is an example of a negative selectable marker which can be used as

a second marker to eliminate cells that carry it. Cells with the HSV tk gene are selectively killed in the presence of gangcyclovir.

Cells are transfected with targeting constructs and then selected for the presence of the first selection marker and the absence of the second. Constructs / DNA are then injected into the blastocyst stage and implanted into pseudopregnant females. Chimeric offspring which are capable of transferring the recombinant genes in their germline are selected, mated and their offspring examined for heterozygous carriers of the recombined genes. Mating of the heterozygous offspring can then be used to generate fully homozygous offspring which constitute HDAC-deficient knock-out mice.

Embodiments of the Invention

- An isolated polynucleotide encoding a histone deacetylase polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95.
- An isolated polynucleotide encoding an amino acid sequence selected from the group consisting of:
    a. an amino acid sequence comprising residues 1009-1069 of SEQ ID NO:87; and
    b. an amino acid sequence comprising residues 720-780 of SEQ ID NO:93.
- An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and SEQ ID NO:96.
- An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
    a. a nucleotide sequence which is at least 60% identical to SEQ ID NO:1;
    b. a nucleotide sequence which is at least 60% identical to SEQ ID NO:12;
    c. a nucleotide sequence which is at least 60% identical to SEQ ID NO:19;

d. a nucleotide sequence which is at least 67.8% identical to SEQ ID NO:88;

e. a nucleotide sequence which is at least 70% identical to SEQ ID NO:94;

f. a nucleotide sequence which is at least 59.8% identical to SEQ ID NO:96;

g. a nucleotide sequence which is at least 94.4% identical to nucleotides 1 to 3207 of SEQ ID NO:88;

h. a nucleotide sequence which is at least 55.4% identical to nucleotides 307 to 1791 of SEQ ID NO:96.

i. a nucleotide sequence comprising nucleotides 1 to 3207 of SEQ ID NO:88;

j. a nucleotide sequence comprising nucleotides 1 to 2340 of SEQ ID NO:94;

k. a nucleotide sequence comprising nucleotides 307 to 1791 of SEQ ID NO:96;

l. a nucleotide sequence comprising nucleotides 4 to 3207 of SEQ ID NO:88 wherein said nucleotides encode amino acids 2 to 1069 of SEQ ID NO:87 lacking the start methionine; and

m. a nucleotide sequence comprising nucleotides 310 to 1791 of SEQ ID NO:96 wherein said nucleotides encode amino acids 2 to 495 of SEQ ID NO:95 lacking the start methionine.

- An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence comprising at least 25 contiguous nucleotides of SEQ ID NO1;

b. a nucleotide sequence comprising at least 25 contiguous nucleotides of SEQ ID NO:12;

c. a nucleotide sequence comprising at least 25 contiguous nucleotides of SEQ ID NO:19;

d. a nucleotide sequence comprising at least 2755 contiguous nucleotides of SEQ ID NO:88;

e. a

nucleotide sequence comprising at least 2160 contiguous nucleotides of SEQ ID NO:94; f. a nucleotide sequence comprising at least 1195 contiguous nucleotides of SEQ ID NO:96; g. a nucleotide sequence comprising at least 183 contiguous nucleotides of SEQ ID NO:88; and h. a nucleotide sequence comprising at least 17 contiguous nucleotides of SEQ ID NO:96.

- An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

a. a nucleotide sequence comprising nucleotides 3024-4467 of SEQ ID NO:88;

b. a nucleotide sequence comprising nucleotides 2156-3650 of SEQ ID NO:94;

c. a nucleotide sequence comprising nucleotides 1174-3391 of SEQ ID NO:96;

d. a nucleotide sequence comprising nucleotides 3024-3207 of SEQ ID NO:88; and

e. a nucleotide sequence comprising nucleotides 1174-1791 of SEQ ID NO:96.

- An primer comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:24-27, SEQ ID NO:28-35, SEQ ID NO:39-46, SEQ ID NO:47-62, SEQ ID NO:65-66, SEQ ID NO:67-74, SEQ ID NO:75-82, and SEQ ID NO:104-105.
- A probe comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:63-64, SEQ ID NO:83-86, SEQ ID NO92, and SEQ ID NO:101-103.
- A cell line comprising the isolated polynucleotide according to any one of the preceding embodiments.
- A gene delivery vector comprising the isolated polynucleotide according to any one of the preceding embodiments.

- An expression vector comprising the isolated polynucleotide according to any one of the preceding embodiments.
- A host cell comprising the expression vector according to any one of the preceding embodiments, wherein the host cell is selected from the group consisting of bacterial, yeast, insect, mammalian, and human cells.
- An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95.
- An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a. an amino acid sequence which is at least 72% identical to SEQ ID NO:2;

b. an amino acid sequence which is at least 79% identical to SEQ ID NO:4;

c. an amino acid sequence which is at least 70% identical to SEQ ID NO:5;

d. an amino acid sequence which is at least 94.2% identical to SEQ ID NO:87;

e. an amino acid sequence which is at least 95% identical to SEQ ID NO:93; and

f. an amino acid sequence which is at least 55.3% identical to SEQ ID NO:95.

- An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a. an amino acid sequence comprising at least 8 contiguous amino acids of SEQ ID NO:2;

b. an amino acid sequence comprising at least 8 contiguous amino acids of SEQ ID NO:4;

c. an amino acid sequence comprising at least 8 contiguous amino acids of SEQ ID NO:5;

d. an amino acid sequence comprising at least 920 contiguous amino acids of SEQ ID NO:87;

e. an amino acid

sequence comprising at least 720 contiguous amino acids of SEQ ID NO:93; and f. an amino acid sequence comprising at least 400 contiguous amino acids of SEQ ID NO:95.

- An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

  a. an amino acid sequence comprising residues 1009-1069 of SEQ ID NO:87; and

  b. an amino acid sequence comprising residues 720-780 of SEQ ID NO:93.

- An isolated fusion protein comprising the isolated polypeptide according to any one of the preceding embodiments.
- An antibody which binds specifically to the isolated polypeptide according to any one of the preceding embodiments, wherein the antibody is selected from the group consisting of polyclonal and monoclonal antibodies.
- An antibody which binds specifically to the isolated fusion protein according to any one of the preceding embodiments.
- An antisense polynucleotide comprising a nucleotide sequence that is complementary to at least 20 contiguous nucleotides of the isolated polynucleotide according to any one of the preceding embodiments.
- An antisense polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:63-64, and SEQ ID NO:83-86.
- An expression vector comprising the antisense polynucleotide according to any one of the preceding embodiments.
- A pharmaceutical composition comprising the monoclonal antibody according to any one of the preceding embodiments, and a physiologically acceptable carrier, diluent, or excipient.
- A pharmaceutical composition comprising the antisense polynucleotide according to any one of the preceding embodiments and a physiologically acceptable carrier, diluent, or excipient.

- A pharmaceutical composition comprising the expression vector according to any one of the preceding embodiments, and a physiologically acceptable carrier, diluent, or excipient.
- A pharmaceutical composition comprising the gene delivery vector according to any one of the preceding embodiments, and a physiologically acceptable carrier, diluent, or excipient.
- A pharmaceutical composition comprising the host cell according to any one of the preceding embodiments, and a physiologically acceptable carrier, diluent, or excipient.
- A pharmaceutical composition comprising the modulating agent according to any one of the following embodiments, and a physiologically acceptable carrier, diluent, or excipient.
- A method of treating cancer comprising administering the pharmaceutical composition according to any one of the preceding embodiments in an amount effective for treating the cancer.

In various aspects, the cancer is selected from the group consisting of bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer, prostate cancer, and melanoma.

In other aspects, the breast cancer is selected from the group consisting of ductal carcinoma *in situ*, intraductal carcinoma lobular carcinoma *in situ*, papillary carcinoma, and comedocarcinoma, adenocarcinomas, and carcinomas, such as infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma.

In further aspects, the prostate cancer is selected from the group consisting of adenocarcinomas and sarcomas, and pre-cancerous conditions, such as prostate intraepithelial neoplasia.

- A method of diagnosing a cancer comprising:

a. incubating the isolated polynucleotide according to any

one of the preceding embodiments with a biological sample under conditions to allow the isolated polynucleotide to amplify a polynucleotide in the sample to produce a amplification product; and

b. measuring levels of amplification product formed in (a), wherein an alteration in these levels compared to standard levels indicates diagnosis of the cancer.

In various aspects, the cancer is selected from the group consisting of bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer, prostate cancer, and melanoma. In other aspects, the breast cancer is selected from the group consisting of ductal carcinoma *in situ*, intraductal carcinoma lobular carcinoma *in situ*, papillary carcinoma, and comedocarcinoma, adenocarcinomas, and carcinomas, such as infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma. In further aspects, the prostate cancer is selected from the group consisting of adenocarcinomas and sarcomas, and pre-cancerous conditions, such as prostate intraepithelial neoplasia.

- A method of diagnosing cancer comprising:

a. contacting the antibody according to any one of the preceding embodiments with a biological sample under conditions to allow the antibody to associate with a polypeptide in the sample to form a complex; and

b. measuring levels of complex formed in (a), wherein an alteration in these levels compared to standard levels indicates diagnosis of the cancer.

In various aspects, the cancer is selected from the group consisting of bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer,

prostate cancer, and melanoma.

In other aspects, the breast cancer is selected from the group consisting of ductal carcinoma *in situ*, intraductal carcinoma lobular carcinoma *in situ*, papillary carcinoma, and comedocarcinoma, adenocarcinomas, and carcinomas, such as infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma.

In further aspects, the prostate cancer is selected from the group consisting of adenocarcinomas and sarcomas, and pre-cancerous conditions, such as prostate intraepithelial neoplasia.

- A method of detecting a histone deacetylase polynucleotide comprising:

  a. incubating the isolated polynucleotide according to any one of the preceding embodiments with a biological sample under conditions to allow the polynucleotide to hybridize with a polynucleotide in the sample to form a complex; and

  b. identifying the complex formed in (a), wherein identification of the complex indicates detection of a histone deacetylase polynucleotide.

- A method of detecting a histone deacetylase polypeptide comprising:

  a. incubating the antibody according to any one of the preceding embodiments with a biological sample under conditions to allow the antibody to associate with a polypeptide in the sample to form a complex; and

  b. identifying the complex formed in (a), wherein identification of the complex indicates detection of a histone deacetylase polypeptide.

- A method of screening test agents to identify modulating agents capable of altering deacetylase activity of a histone deacetylase polypeptide comprising:

  a. contacting the isolated polypeptide according to any one of the preceding embodiments with test agents under conditions to allow

the polypeptide to associate with one or more test agents; and

b. selecting test agents that alter the deacetylase activity of the polypeptide, whereby this alteration indicates identification of modulating agents. In various aspects, the modulating agents are selected from the group consisting of antagonists and inhibitors of histone deacetylase activity. In other aspects, the modulating agents are selected from the group consisting of agonists or activators of histone deacetylase activity.

- A method for screening test agents to identify modulating agents which inhibit or antagonize deacetylation activity of a histone deacetylase, comprising:

a. combining an isolated polypeptide according any one of the preceding embodiments having a histone deacetylase activity with a histone deacetylase substrate and a test agent in a reaction mixture; and

b. determining the conversion of the substrate to product; wherein a statistically significant decrease in the conversion of the substrate in the presence of the test agent indicates identification of a modulating agent which inhibits or antagonizes the deacetylation activity of histone deacetylase.

- A method for screening test agents to identify modulating agents that inhibit or antagonize interaction of histone deacetylase with a histone deacetylase binding protein, comprising:

a. combining the isolated polypeptide according any one of the preceding embodiments having a histone deacetylase activity with the histone deacetylase binding protein and a test agent in a reaction mixture; and

b. detecting the interaction of the polypeptide with the histone deacetylase binding protein to form a complex; wherein a statistically significant decrease in the interaction of the polypeptide and protein in the presence of the test agent indicates identification of a modulating agent which inhibits or antagonizes interaction of the histone deacetylase

polypeptide with the histone deacetylase binding protein.

In various aspects, one or both of the histone deacetylase polypeptide and the histone deacetylase binding protein is a fusion protein. In other aspects, at least one of the histone deacetylase polypeptide and the histone deacetylase binding protein comprises a detectable label for detecting the formation of the complex. In a further aspect, the interaction of the histone deacetylase polypeptide and the histone deacetylase binding protein is detected in a two-hybrid assay system.

- A method of screening a library of molecules or compounds to identify at least one molecule or compound therein which specifically binds to a histone deacetylase polynucleotide, comprising:

  a. combining the isolated polynucleotide according to any one of the preceding embodiments with a library of molecules or compounds under conditions to allow specific binding of the polynucleotide to at least one of the molecules or compounds; and b. detecting the specific binding in (a), thereby identifying a molecule or compound which specifically binds to the histone deacetylase polynucleotide. In various aspects, the library comprises molecules selected from the group consisting of selected from the group consisting of DNA molecules, RNA molecules, artificial chromosomes, PNAs, peptides, and polypeptides. In one aspect, the detecting is performed by the use of high throughput screening.

- A method of treating a disease or disorder associated with abnormal cell growth or proliferation in a mammal comprising administrating the antagonist or inhibitor of histone deacetylase polypeptide according to any one of the preceding embodiments in an amount effective to treat the disease or disorder.

  In various aspects, the disease or disorder is selected from neoplasms, tumors and cancers.

- A method of treating a disease or disorder associated with abnormal cell growth or proliferation in a mammal comprising administrating the antisense polynucleotide according to any one of the preceding embodiments in an amount effective to treat the disease or disorder.

In various aspects, the disease or disorder is selected from neoplasms, tumors and cancers.

- A method of modulating one or more of cell growth or proliferation, cell differentiation, or cell survival of a eukaryotic cell, comprising combining the cell with an effective amount of a modulating agent that alters the deacetylase activity of a histone deacetylase polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95, and thereby modulating the rate of one or more of cell growth or proliferation, cell differentiation, or cell survival of the eukaryotic cell, relative to the effect on the eukaryotic cells in the absence of the modulating agent.

## EXAMPLES

The Examples below are provided to illustrate the subject invention and are not intended to limit the invention in any way.

### EXAMPLE 1: IDENTIFICATION OF NOVEL HDAC GENE FRAGMENTS

Gene fragments encoding the novel HDAC (HDAL) polypeptides of this invention were identified by a combination of the following methods. Homology-based searches using the TBLASTN program (S.F. Altschul et al., 1997, *Nucl. Acids Res.*, 25(17):3389-3402) were performed to compare known histone deacetylases with human genomic (gDNA) and EST sequences. EST or gDNA sequences having significant homology to one or more of phosphatases (expect score less than or equal to $1x10^{-3}$) were retained for further analysis.

Hidden Markov Model (HMM) searches using PFAM motifs (listed in Table 2) (A. Bateman et al., 1999, *Nucleic Acids Research*, 27:260-262 and E.L. Sonnhammer et al., 1997, *Proteins*, 28(3):405-420) to search human genomic sequence using the Genewise program. EST or gDNA sequences

having a significant score (greater than or equal to 10) with any of the following motifs were retained for further analysis.

HMM searches using PFAM motifs (listed in Table 1) to search predicted protein sequences identified by GENSCAN analysis of human genomic sequence (C. Burge and S. Karlin, 1997, *J. Mol. Biol.*, 268(1):78-94). gDNA sequences having a significant score (greater than or equal to 10) with any of the following motifs were retained for further analysis.

Table 1: PFAM motifs used to identify histone deacetylases

| Motif Name | PFAM Accession # | Description |
|---|---|---|
| Hist_deacetyl | PF00850 | Histone deacetylase family (length 342) |

Once a bacterial artificial chromosome (BAC) encoding a novel histone deacetylase-like protein was identified by any of the methods listed above, its predicted protein sequence was used to identify the most closely related known histone deacetylase using the BLASTP program(NCBI). This known protein was used as the query for a GenewiseDB search of the original BAC and all nearby BACs (identified by the Golden Path tiling map, UCSC). The results were used to identify additional potential exons, intron/exon boundaries, partial transcript cDNA sequence and partial predicted protein sequence for the novel HDAC gene. The Primer3 program (S. Rozen et al., 1998, 0.6 Ed., Whitehead Institute Center for Genomic Research, Cambridge, MA) was used to design PCR primers within single exons and between adjacent exons and to design antisense 80mer probes for use in isolating cDNA clones.

**EXAMPLE 2: ANALYSIS OF HDACs**

Enzymatic Activity Measurements

Constructs representing the open reading frames of the identified novel sequences are engineered in frame with c-MYC or FLAG epitopes using commercially available mammalian expression vectors. These plasmids are transfected into HEK293 or COS7 cells and novel HDAC protein expression are analyzed by Western .blot analysis of protein lysates from the transfectants using anti-MYC epitope or anti-FLAG epitope antibodies.

MYC or FLAG tagged-HDAC proteins are immunoprecipitated from the lysates and incubated with {$^3$H} acetate- or fluorescent-labeled acetylated proteins. Release of {$^3$H} acetate or decrease in fluorescent signal intensity is used to establish the activity of the putative HDACs. The effects of pan-HDAC chemical inhibitors on the enzymatic activity of the novel HDACs is also assessed and compared with the activity of known HDAC proteins and their inhibition with these chemical agents.

Transcriptional Assays

HDAC proteins have been shown to positively or negative regulate transcriptional pathways. The ability of the novel HDAC proteins to repress or activate the constitutive or regulated activity of transcriptional reporter plasmids is assessed. These assays are performed using transient transfections of mammalian expression constructs encoding the novel HDAC proteins with reporter plasmid constructs of containing response elements of specific transcriptional pathways (e.g., p53, AP1, androgen receptor, LEF1/TCF4), a minimal promoter and a reporter gene product (e.g., alkaline phosphatase, luciferase, green fluorescent protein).

Alternatively, the novel HDACs are transfected into cell lines engineered to stably express these transcriptional reporter plasmids. Because the consequence of HDAC expression could be inhibitory or stimulatory, the effects of the novel HDAC proteins on these transcriptional responses are monitored in the presence and absence of activators of the pathway. Similar to enzymatic activity measurements, pan-inhibitors of the known HDACs are also examined to establish the enzymatic activity of the novel HDAC gene products as protein deacetylases.

Expression Analysis

Initial insights into the role of the novel HDACs in normal physiology and disease states is assessed by a variety of expression analyses. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using primers specific to the novel sequences is implemented to evaluate the expression of novel HDAC mRNA in a variety of normal cell lines and tissue as well as a spectrum of human tumor cell lines. Expression profiles of novel

HDACs are confirmed using Northern blot analysis or ribonuclease protection assays.

In addition, tissue arrays containing a variety of patient organ samples and arrays of malignant tissue are evaluated by *in situ* hybridization to gain further insights into the association of the novel HDAC proteins with particular physiological responses and in neoplasia.

Subcellular Localization

The subcellular localization of MYC- or FLAG-tagged novel HDAC proteins is determined upon ectopic expression in mammalian cells. Cells are fixed, permeabilized and incubated with anti-MYC or anti-FLAG antibodies to detect expressed protein. The localization of tagged proteins is then detected using CY3 or FITC-conjugated secondary antibodies and visualized by fluorescent microscopy. These studies can determine if the assayed HDACs deacetylate nuclear or cytoplasmic protein substrates.

**EXAMPLE 3: OLIGONUCLEOTIDES FOR THE ISOLATION OF HDACs**

BMY_HDAL1

Based on the predicted gene structure of BMY_HDAL1, the Primer3 program designed the following PCR primers and probe oligos for isolation of cDNAs. Table 2 presents single exon primers and probes for BMY_HDAL1 cDNA isolation. Table 3 presents multiple exon primers for BMY_HDAL1 cDNA isolation. Table 4 presents BMY_HDAL1 capture oligonucleotides. As shown below in Table 5, a separately designed primer set was used to test for BMY_HDAL1 expression using a cDNA pool from human placenta and the following human tumor cell lines including Caco-2, LS174-T, MIP, HCT-116, A2780, OVCAR-3, HL60, A431, Jurkat, A549, PC3 and LnCAP cells.

BMY_HDAL2

Based on the predicted gene structure of BMY_HDAL2, the Primer3 program designed the following PCR primers and probe oligonucleotides for isolation of cDNAs. BMY_HDAL2 single exon primers and probes are shown in Table 6. Multiple exon primers for BMY-HDAL2 cDNA isolation are shown in Table 7. BMY_HDAL2 capture oligonucleotides are shown in Table 8. As shown in Table 9, a separately designed primer set was used to test for

BMY_HDAL2 expression using a cDNA pool from human placenta and the following human tumor cell lines: Caco-2, LS174-T, MIP, HCT-116, A2780, OVCAR-3, HL60, A431, Jurkat, A549, PC3 and LnCAP cells.

BMY_HDAL3

Based on the predicted gene structure of BMY_HDAL3, the Primer3 program designed the following PCR primers and probe oligonucleotides for isolation of cDNAs. For BMY_HDAL3, the following primer sets were designed from the AC002410 sequence using Primer3. Single exon primers for the novel BMY-HDAL3 isolation are shown in Table 10. Multiple exon primers for BMY_HDAL3 isolation are presented in Table 11. BMY_HDAL3 capture oligonucleotides are shown in Table 12.

## Table 2

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Product Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL1 exon 1 | 1 | 118 | 16, 20 | ccttgatgctgaaacaccag (SEQ ID NO:24) | 59.3 | 133, 21 | tcacatttatttagcagccca (SEQ ID NO:25) | 58.3 |
| BMY_HDAL1 exon 1 | 2 | 119 | 16, 20 | ccttgatgctgaaacaccag (SEQ ID NO:26) | 59.3 | 134,22 | ctcacatttatttagcagccca (SEQ ID NO:27) | 59.3 |

## Table 3

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Product Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL1 exons 1_2 | 1 | 148 | 67, 20 | agcatgctggacgaatacag (SEQ ID NO:28) | 58.9 | 234, 20 | ttggtgccatacaacagtga (SEQ ID NO:29) | 58.5 |
| BMY_HDAL1 exons 1_2 | 2 | 199 | 16, 20 | ccttgatgctgaaacaccag (SEQ ID NO:30) | 59.3 | 234, 20 | ttggtgccatacaacagtga (SEQ ID NO:31) | 58.5 |
| BMY_HDAL1 exons 2_3 | 1 | 110 | 60, 20 | tcactgttgtatggcaccaa (SEQ ID NO:32) | 58.5 | 189, 20 | ccaagtccaccacaaggtaa (SEQ ID NO:33) | 58.5 |
| BMY_HDAL1 exons 2_3 | 2 | 104 | 60, 20 | tcactgttgtatggcaccaa (SEQ ID NO:34) | 58.5 | 183, 20 | ccaccacaaggtaatgagga (SEQ ID NO:35) | 58.4 |

## Table 4

| Oligo | | Capture Probe | |
|---|---|---|---|
| Template | Number | Start, Size | Sequence (ANTISENSE) |
| BMY_HDAL2 exon 1 | 1 | 36, 77 | gtttcttgcagtcgtgaccagatactctgtattcgtccagcatgctcagggtgggtggtggaattgccacaaacgca (SEQ ID NO:36) |

## Table 5

| HDAL Gene | 5'-oligo primer sequence (5'-3') | 3'-oligo primer sequence (5'-3') | Predicted Product | Product observed |
|---|---|---|---|---|
| HDAL1 | ggaattgcctatgacccttga (SEQ ID NO:37) | tgtacttaccccaagtccaccaca (SEQ ID NO:38) | 316 nt | yes |

## Table 6

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Pro-duct Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL2 exon 1 | 1 | 102 | 2, 20 | ggacagtgacaccatttgga (SEQ ID NO:39) | 59.4 | 103, 19 | agctctcctgaggccactt (SEQ ID NO:40) | 59.1 |
| BMY_HDAL2 exon 1 | 2 | 100 | 2, 20 | ggacagtgacaccatttgga (SEQ ID NO:41) | 59.4 | 101, 19 | ctctcctgaggccactttg (SEQ ID NO:42) | 58.5 |
| BMY_HDAL2 exon 4 | NA | | | | | | | |
| BMY_HDAL2 exon 5 | 1 | 103 | 10, 20 | gccttggagaagggtacaat (SEQ ID NO:43) | 58.1 | 112, 23 | gaaagaagtaccaacctgaatgc (SEQ ID NO:44) | 59.2 |
| BMY_HDAL2 exon 5 | 2 | 102 | 10, 20 | gccttggagaagggtacaat (SEQ ID NO:45) | 58.1 | 111, 22 | aaagaagtaccaacctgaatgc (SEQ ID NO:46) | 57.4 |

**Table 7**

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Product Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL2 exons 1-2 | 1 | 157 | 2, 20 | ggacagtgacaccatttgga (SEQ ID NO:47) | 59.4 | 178, 2 | tgtggattcttcagcgtgat (SEQ ID NO:48) | 59.2 |
| BMY_HDAL2 exons 1-2 | 2 | 126 | 2, 20 | ggacagtgacaccatttgga (SEQ ID NO:49) | 59.4 | 147, 20 | ctcacaacagcaaacccatt (SEQ ID NO:50) | 58.6 |
| BMY_HDAL2 exons 2-3 | 1 | 107 | 0, 20 | aatgggtttgctgttgtgag (SEQ ID NO:51) | 58.6 | 126, 20 | tctctcaagtatttggcggt (SEQ ID NO:52) | 57.4 |
| BMY_HDAL2 exons 2-3 | 2 | 108 | 0, 20 | aatgggtttgctgttgtgag (SEQ ID NO:53) | 58.6 | 127, 20 | gtctctcaagtatttggcgg (SEQ ID NO:54) | 57.4 |
| BMY_HDAL2 exons 3-4 | 1 | 130 | 23, 20 | ttgcaattaccgccaaatac (SEQ ID NO:55) | 58.6 | 172, 20 | gaaatgtacaggatgctggg (SEQ ID NO:56) | 58.0 |
| BMY_HDAL2 exons 3-4 | 2 | 131 | 22, 20 | gttgcaattaccgccaaata (SEQ ID NO:57) | 58.561 | 172, 20 | gaaatgtacaggatgctggg (SEQ ID NO:58) | 58.019 |
| BMY_HDAL2 exons 4-5 | 1 | 105 | 45, 20 | cccagcatcctgtacatttc (SEQ ID NO:59) | 58.019 | 169, 20 | attgtacccttctccaaggc (SEQ ID NO:60) | 58.121 |
| BMY_HDAL2 exons 4-5 | 2 | 113 | 69, 20 | catcgctatgatgaagggaa (SEQ ID NO:61) | 58.671 | 201, 18 | ggatcaaggccacctgtc (SEQ ID NO:62) | 58.969 |

## Table 8

| Set | | Capture Probe | |
|---|---|---|---|
| Template | Oligo Number | Start, Size | Sequence (ANTISENSE) |
| BMY_HDAL2 exon 1 | No oligo | | |
| BMY_HDAL2 exon 4 | 1 | 23, 80 | tgccagggaaaaagttcccttcatcatagcgatggagtgaaatgtacaggatgctggggtcagcataaaaggcctgctgg (SEQ ID NO:63) |
| BMY_HDAL2 exon 4 | 2 | 19, 79 | gggaaaaagttcccttcatcatagcgatggagtgaaatgtacaggatgctggggtcagcataaaaggcctgctgggtac (SEQ ID NO:64) |

## Table 9

| HDAL Gene | 5'-oligo primer sequence (5'-3') | 3'-oligo primer sequence (5'-3') | Predicted Product | Product observed |
|---|---|---|---|---|
| HDAL2 | gtggacagtgacaccatttgga (SEQ ID NO:65) | ggagaaagaagtaccaacctgaatgctt (SEQ ID NO:66) | 489 nt | yes |



## Table 10

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Product Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL3 exon 1 | 1 | 100 | 18, 20 | gtggccaaagagtttgatcc (SEQ ID NO:67) | 60 | 117, 20 | ttgccgtcactttgtaccct (SEQ ID NO:68) | 60 |
| BMY_HDAL3 exon 1 | 2 | 100 | 18, 20 | gtggccaaagagtttgatcc (SEQ ID NO:69) | 60 | 117, 19 | ttgccgtcactttgtaccc (SEQ ID NO:70) | 59 |
| BMY_HDAL3 exon 2 | 1 | 120 | 4, 20 | tggtcatttgacgaagcaat (SEQ ID NO:71) | 59 | 123, 20 | agaagggcatttacacaggc (SEQ ID NO:72) | 59 |
| BMY_HDAL3 exon 2 | 2 | 119 | 4, 20 | tggtcatttgacgaagcaat (SEQ ID NO:73) | 59 | 122, 20 | gaagggcatttacacaggct (SEQ ID NO:74) | 59 |

## Table 11

| Primer Set | | | Left Primer | | | Right Primer | | |
|---|---|---|---|---|---|---|---|---|
| Template | Set | Product Size | Start, Length | Sequence | Tm | Start, Length | Sequence | Tm |
| BMY_HDAL3 exons 1-2 | 1 | 147 | 95, 20 | aggagggtacaaagtgacgg (SEQ ID NO:75) | 59 | 261, 20 | agggcatttacacaggcttc (SEQ ID NO:76) | 59 |
| BMY_HDAL3 exons 1-2 | 2 | 146 | 95, 20 | aggagggtacaaagtgacgg (SEQ ID NO:77) | 59 | 260, 20 | gggcatttacacaggcttct (SEQ ID NO:78) | 59 |
| BMY_HDAL3 exons 2-3 | 1 | 160 | 25, 20 | gatgacattggctgatggac (SEQ ID NO:79) | 59 | 204, 20 | agcattcatattcgggcttt (SEQ ID NO:80) | 59 |
| BMY_HDAL3 exons 2-3 | 2 | 181 | 4, 20 | tggtcatttgacgaagcaat (SEQ ID NO:81) | 59 | 204, 20 | agcattcatattcgggcttt (SEQ ID NO:82) | 59 |



## Table 12

| Set | | Capture Probe | |
|---|---|---|---|
| Template | Set | Start, Size | Sequence (ANTISENSE) |
| BMY_HDAL3 exon 1 | 1 | 32, 80 | tcactttgtaccctcctagaggaggggtgtggccttccaatgcatcaaatccagcagatactaagaccatgtctggatca (SEQ ID NO:83) |
| BMY_HDAL3 exon 1 | 2 | 19, 80 | tcctagaggaggggtgtggccttccaatgcatcaaatccagcagatactaagaccatgtctggatcaaactctttggcca (SEQ ID NO:84) |
| BMY_HDAL3 exon 2 | 1 | 27, 80 | ggcttctgatgcatcacagatggctgtgagatcatgtcctccttctagagccaacaccacacgtccatcagccaatgtca (SEQ ID NO:85) |
| BMY_HDAL3 exon 2 | 2 | 27, 80 | ggcttctgatgcatcacagatggctgtgagatcatgtcctccttctagagccaacaccacacgtccatcagccaatgtca (SEQ ID NO:86) |

## EXAMPLE 4: COMPLEMENTARY POLYNUCLEOTIDES

Antisense molecules or nucleic acid sequence complementary to an HDAC protein-encoding sequence, or any part thereof, can be used to decrease or to inhibit the expression of naturally occurring HDAC. Although the use of antisense or complementary oligonucleotides comprising about 15 to 35 base-pairs is described, essentially the same procedure is used with smaller or larger nucleic acid sequence fragments. An oligonucleotide based on the coding sequence of an HDAC polypeptide or peptide, for example, as shown in FIG. 1, FIG. 5, FIG. 10, FIGS. 15A-15C, FIGS. 20A-20C, and FIGS. 21A-21B, and as depicted in SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, for example, is used to inhibit expression of naturally occurring HDAC. The complementary oligonucleotide is typically designed from the most unique 5' sequence and is used either to inhibit transcription by preventing promoter binding to the coding sequence, or to inhibit translation by preventing the ribosome from binding to an HDAC protein-encoding transcript.

Using a portion SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, for example, an effective antisense oligonucleotide includes any of about 15-35 nucleotides spanning the region which translates into the signal or 5' coding sequence of the HDAC polypeptide. Appropriate oligonucleotides are designed using OLIGO 4.06 software and the HDAC coding sequence (e.g., SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96).

## EXAMPLE 5: NORTHERN BLOT ANALYSIS FOR HDACs

Northern Blot analysis is used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNA from a particular cell or tissue type has been bound (See, J. Sambrook et al., *supra*). Analogous computer techniques using BLAST (S.F. Altschul, 1993, *J. Mol. Evol.*, 36:290-300 and S.F. Altschul et al., 1990, *J. Mol. Evol.*, 215:403-410) are used to search for identical or related molecules in nucleotide databases, such as GenBank or the LIFESEQ database (Incyte Pharmaceuticals). This analysis is much more rapid and

less labor-intensive than performing multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as being exact (identical) or homologous.

The basis of the search is the product score, which is defined as follows: (% sequence identity x maximum BLAST score) / 100. The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules. The results of Northern analysis are reported as a list of libraries in which the transcript encoding HDAC polypeptides occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times that a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences that are examined in the cDNA library.

**EXAMPLE 6: MICROARRAYS FOR ANALYSIS OF HDACs**

For the production of oligonucleotides for a microarray, an HDAC sequence, e.g., a novel HDAC having SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, or SEQ ID NO:96, for example, is examined using a computer algorithm which starts at the 3' end of the nucleotide sequence. The algorithm identifies oligomers of defined length that are unique to the gene, have a GC content within a range that is suitable for hybridization and lack predicted secondary structure that would interfere with hybridization. The algorithm identifies specific oligonucleotides of 20 nucleotides in length, i.e., 20-mers. A matched set of oligonucleotides is created in which one nucleotide in the center of each sequence is altered. This process is repeated for each gene in the microarray, and double sets of 20-mers are synthesized in the presence of fluorescent or radioactive nucleotides and arranged on the surface of a substrate. When the substrate

is a silicon chip, a light-directed chemical process is used for deposition (WO 95/11995, M. Chee et al.).

Alternatively, a chemical coupling procedure and an ink jet device is used to synthesize oligomers on the surface of a substrate. (WO 95/25116, J.D. Baldeschweiler et al.). As another alternative, a "gridded" array that is analogous to a dot (or slot) blot is used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using, for example, a vacuum system, or thermal, UV, mechanical, or chemical bonding techniques. A typical array may be produced by hand, or by using available materials and equipment, and may contain grids of 8 dots, 24 dots, 96 dots, 384 dots, 1536 dots, or 6144 dots. After hybridization, the microarray is washed to remove any non-hybridized probe, and a detection device is used to determine the levels and patterns of radioactivity or fluorescence. The detection device may be as simple as X-ray film, or as complicated as a light scanning apparatus. Scanned fluorescent images are examined to determine degree of complementarity and the relative abundance/expression level of each oligonucleotide sequence in the microarray.

**EXAMPLE 7: PURIFICATION OF HDAC POLYPEPTIDES**

Naturally occurring or recombinant HDAC polypeptide is substantially purified by immunoaffinity chromatography using antibodies specific for an HDAC polypeptide, or a peptide derived therefrom. An immunoaffinity column is constructed by covalently coupling anti-HDAC polypeptide antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Medium containing HDAC polypeptide is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of the HDAC polypeptide (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HDAC polypeptide binding (e.g., a buffer of pH 2-3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HDAC polypeptide is collected.

**EXAMPLE 8: IDENTIFICATION OF MOLECULES THAT INTERACT WITH HDAC POLYPEPTIDES**

HDAC polypeptides, or biologically active fragments thereof, are labeled with $^{125}$I Bolton-Hunter reagent (Bolton et al., 1973, *Biochem. J.*, 133:529). Candidate molecules previously arrayed in wells of a multi-welled plate are incubated with the labeled HDAC polypeptide, washed, and any wells having labeled HDAC polypeptide-candidate molecule complexes are assayed. Data obtained using different concentrations of HDAC polypeptide are used to calculate values for the number, affinity and association of an HDAC polypeptide with the candidate molecules.

Another method suitable for identifying proteins, peptides or other molecules that interact with an HDAC polypeptide include ligand binding assays such as the yeast-two hybrid system as described hereinabove.

**EXAMPLE 9: IDENTIFICATION AND CLONING OF HDAC9c**

Bioinformatic searches of the assembled human genome sequence were performed using a conserved consensus sequence derived from the catalytic domain of class I and class II HDACs. Three gene fragments (HDAL1, HDAL2, HDAL3) were identified from the assembled sequence of human chromosome 7q36 that encoded amino acids sequence with homology to class II HDACs. Biotinylated single stranded oligonucleotides representing unique sequences from these predicted gene fragments of the following sequence were prepared:

HDAL1, 5-gtttcttgcagtcgtgaccagatactctgattcgtccagcatgctcagggt gggtgggtggaattgccacaaacgca (SEQ ID NO:101);

HDAL2, 5'-tgccagggaaaaagt tcccttcatcatagcgatggagtgaaatgtaca ggatgctggggtcagcataaaaggcctgctgg (SEQ ID NO:102); and

HDAL3, 5' tgatccagacatggtcttagtatctgctggatttgatgcattggaaggcca caccctcctctaggagggtacaaagtga (SEQ ID NO:103).

The biotinylated oligonucleotides were hybridized to fractions of cDNA prepared from human placenta, and positive sequences were identified by PCR. Three of the clones identified (HDACX1A, HDACX2A, and HDACX3A) contained overlapping cDNAs that showed sequence identity to the predicted

gene fragments. These cDNAs encoded a novel sequence, designated HDAC9c (FIGS. 15A-15C), that shared homology to class II HDACs. A full length HDAC9c construct was prepared by combining a 1.3 kb *Bam*HI-*Pst*I fragment from the HDACX2A clone with a 3.5 kb *Pst*I-*Not*I fragment from the HDACX3A. These fragments were ligated into mammalian expression vectors pcDNA3.1 and pcDNA4.0. The resulting constructs were evaluated by DNA sequencing to confirm the identity of the inserts. The HDAC9c pcDNA3.1 construct was deposited at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 on June 12, 2002 under ATCC Accession No. _____ according to the terms of the Budapest Treaty.

Three fragments that encoded homology to class II HDACs were identified from the assembled sequence of human chromosome 7q36. Subsequent cDNA cloning bioinformatics analysis revealed that these gene fragments encoded a single class II HDAC, comprising a protein of 1147 amino acids. This sequence was provisionally designated as HDAC-9, and later renamed HDAC9c. During the course of this work, similar sequences were reported by Zhou et al. (2001, *Proc. Natl. Acad. Sci. USA* 98:10572-7), including two isoforms related to class II HDAC proteins. Sequence alignments revealed the HDAC-9 sequence was closely related to the previously identified HDAC9 sequences (GenBank Accession Nos. AY032737 and AY032738). However, the published sequences lacked a large portion of the C-terminal domain common to known class HDAC proteins (FIGS. 15D-15F).

One of the HDAC9 isoforms (HDAC9a, (GenBank Accession No. AY032737) lacked ~ 185 C-terminal amino acids compared to other HDAC family members. Another isoform of HDAC9 (HDAC9, (GenBank Accession No. AY032738) lacked approximately 65 C-terminal amino acids compared to other HDAC family members. In contrast to these sequences, the HDAC9c sequence, also designated as HDAC-X, contained more than 50 additional amino acids at its C-terminus (FIGS. 15D-15F). The HDAC9c sequence was deemed to represent the full-length version of HDAC9. Notably, HDAC9c

contained an LQQ sequence motif at positions 123-125. This motif was missing in the HDAC9 C-terminal truncated isoforms, but was conserved in other HDAC family members. Thus, the LQQ sequence motif may be important for the function of the HDAC9c protein. No other motifs were identified by PFAM analysis (A. Bateman et al., 2002, *Nucl. Acids Res.* 30:276-80).

**EXAMPLE 10: EXPRESSION PROFILING FOR HDAC9**

To determine the distribution of HDAC9 in adult normal tissues, the expression profile of HDAC9 was examined by Northern blot analysis. Northern blotting was performed as described (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition). Tissue samples were obtained from CLONTECH (Palo Alto, CA). The probe for Northern blotting was derived from nucleotides 2917-3211 of HDAC9c (FIG. 16D; SEQ ID NO:92). Two > 8.0 kb HDAC9 transcripts were detected at low levels in brain, skeletal muscle, stomach, and trachea tissue (FIG. 16A). Upon longer exposure, HDAC9 mRNA was also detected in mammary gland and prostate tissue (FIG. 16A).

Given the low level of expression in normal tissues, experiments were performed to determine the expression of HDAC9 in human tumor cell lines. HDAC9 mRNA expression levels were evaluated by quantitative PCR analysis on first-strand cDNA prepared from a variety of human tumor cell lines (ATCC, Rockville, MD). HDAC9 levels were normalized to GAPDH mRNA levels within the samples, and RNA levels were quantified using the fluorophore SYBR green. For amplification, HDAC9 primers were used: forward primer 5'-gtgacaccatttggaatgagctac (SEQ ID NO:104); and reverse primer 5'ttggaagccagctcgatgac (SEQ ID NO:105). HDAC9 expression was found to be elevated in ovarian, breast, and certain lung cancer cell lines (FIG. 16B). In contrast, HDAC9 was poorly expressed in tumor cell lines derived from colon tumor specimens (FIG. 16B).

To confirm these results, nuclease protection experiments were performed on RNAs isolated from select tumor cell displaying a range of HDAC9 expression. Nuclease protection was performed using $^{35}$S-labeled

UTP as a radioactive precursor for a in accordance with published methods (Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd Edition). The riboprobe sequence was derived from nucleotides 2917-3211 in HDAC9c (FIG. 16D; SEQ ID NO:92). Brain tissue was included as a control to show normal tissue expression levels. The profile of HDAC9 expression observed by quantitative RT-PCR was confirmed by nuclease protection (i.e., A2780 > MDA-MB453 > MCF7; FIG. 16C). The pervasive expression of HDAC9 in tumor cell lines of diverse origin, and the low level expression of HDAC9 in normal adult tissue, suggested that the expression of this gene was regulated in tumor progression.

**EXAMPLE 11: *IN SITU* HYBRIDIZATION TO ANALYZE HDAC9 EXPRESSION**

To further analyze the upregulation of HDAC9 in tumor cells, a variety of human tumor and normal tissue specimens were subjected to *in situ* hybridization using an HDAC9 antisense riboprobe and tissue microarrays. A $^{35}$S-labeled cRNA riboprobe was prepared from a 295 bp cDNA fragment from the HDAC9 coding region (FIG. 16D; SEQ ID NO:92). This fragment encoded the most divergent region of the HDAC9 protein. The riboprobe was hybridized to paraffin-embedded clinical tissue specimens derived from normal or cancerous tissues, and processed by standard procedures (Lorenzi et al., 1999, *Oncogene* 18:4742-4755). Hybridized sections were incubated for 3 to 6 weeks, and the level and localization of HDAC9 staining was evaluated by microscopy. Staining levels were quantified by a board-certified pathologist.

HDAC9 mRNA levels were generally below the limit of detection (staining level = 0) in normal tissues, including breast, kidney, testis, and liver tissues. Low to moderate levels of HDAC9 mRNA (staining level = 1-2) were detected in lymph node, brain, adrenal gland, pancreas, bladder, lung, and gastric tissues (data not shown). Normal breast and prostate tissue showed average staining levels of 0 and 1, respectively (FIGS. 17A-17C). A dramatic increase in HDAC9 mRNA expression was detected in breast tumor (average staining level = 2-3) and prostate tumor (average staining level = 2) tissues

(FIGS. 17A-17C). Preliminary data also showed increased expression of HDAC9 in endometrial and ovarian tumors. Thus, HDAC9 was expressed at very low levels in normal adult peripheral tissues, but was overexpressed in a variety of tumors, including breast and prostate adenocarcinomas. This suggested that HDAC9 expression correlated with the progression of breast and prostate tumors.

**EXAMPLE 12: EFFECT OF HDAC9c ON CELLULAR TRANSFORMATION**

Results of the experiments, above, indicated that elevated HDAC9c expression was associated with certain tumor cells. To further investigate its involvement in tumorogenesis, HDAC9c was evaluated for its ability to morphologically transform mouse fibroblasts. HDAC9c in pcDNA3.1 was introduced by calcium phosphate transfection into 1.5 x $10^5$ NIH/3T3 cells (ATCC, Rockville, MD) in duplicate at 1.0 µg/10 cm plate. One set of cultures received growth medium (DMEM containing 5% calf serum) while the parallel culture received growth medium containing 750 µg/ml of G418 to develop stable clonal populations.

After 10-14 days in culture, unselected plates were stained with Geimsa (Sigma-Aldrich, St. Louis, MO), and morphologically transformed foci were visualized. Selected clones were examined for growth in soft agar at $10^5$, $10^4$, or $10^3$ cells/15 mm well following standard protocols. After 2-3 weeks in culture, colonies were visualized by microscopy and tetrazolium violet staining. HDAC9c transfectants produced some foci in monolayer culture (data not shown). However, the response was not robust, suggesting that higher levels HDAC9c expression levels were required to transform NIH/3T3 cells.

HDAC9c transfectants were also evaluated for anchorage-independent growth. NIH/3T3 cells stably transfected with HDAC9c or FGF8 constructs, or vector alone, were suspended in soft agar containing growth medium and cultured for 2-3 weeks. FGF8 is a cDNA that potently transforms NIH/3T3 through autocrine stimulation of endogenous FGF receptors (Lorenzi et al., 1995, Oncogene 10:2051-2055). In vector transfectants, very few colonies greater than 50 µm in diameter were observed after three weeks in culture

(FIG. 18). In contrast, FGF8 transfectants produced several colonies greater than 50 μ□m after three weeks (FIG. 18). HDAC9c transfectants also produced significant colony growth compared to vector transfectants, but less than that observed for FGF8 transfectants (FIG. 18). These results suggested that overexpression of HDAC9c induced an oncogenic phenotype in mouse fibroblasts.

**EXAMPLE 13: EFFECT OF HDAC9c ON THE ACTIN CYTOSKELETON**

Changes in the actin cytoskeleton often accompany the transformed phenotype of cells expressing oncogenes such as Ras, Rho, or src. In general, gene products that affect cell adhesion or motility are associated with changes in the actin cytoskeleton. To investigate whether the transformation induced by HDAC9c was associated with changes in the cytoskeletal architecture, NIH/3T3 transfectants expressing HDAC9c were subjected to fluorescent staining with TRITC-conjugated phalloidin to visualize filamentous actin (F-actin).

In these experiments, a HDAC4 construct was used as a control. For the control construct, full-length HDAC4 cDNA was amplified by RT-PCR from first-strand cDNA based on the sequence reported by Grozinger et al. (*Proc. Natl. Acad. Sci. USA* 96:4868-4873), and cloned into pcDNA3.1. Mass-selected stable NIH/3T3 clones of HDAC9c (in pcDNA3.1), Ras, HDAC4, or vector alone, were plated in 8 well chamber slides in duplicate and allowed to adhere overnight in growth medium (DMEM high glucose containing 10% calf serum). Cells were subsequently serum-starved for 18 hours and one set was stimulated with 10% calf serum for 15 minutes. The cultures were fixed for 30 minutes in 4% paraformaldehyde, permeabilized in 0.02% Triton-X100, and incubated with TRITC or FITC conjugated phalloidin (Sigma, St. Louis, MO) for 2 hours. Filamentous actin was visualized by fluorescence microscopy, and images were captured with a digital camera.

In parental NIH/3T3 cells (data not shown) or vector transfectants, low levels of F-actin stress fiber formation were observed following serum starvation for 18 hours (FIG. 19). Stimulation of these cells for 15 minutes with serum promoted an extensive stress fiber network (FIG. 19), indicating

that the extracellular signals regulating these pathways were intact in these cells. A dramatic increase in stress fiber content and organization was observed in serum starved HDAC9c-expressing cells (FIG. 19), indicating that that expression of HDAC9c was sufficient to induce reorganization of the actin cytoskeleton. In contrast, no stress fiber formation was observed in serum starved NIH/3T3 cells expressing the HDAC4 protein (FIG. 19). These results suggested that induction of actin stress fiber formation underlay the transformed phenotype associated with expression of HDAC9c.

Conclusion

Inhibitors of HDAC activity are involved in the regulation of cellular proliferation, apoptosis, and differentiation of a variety of cell types. However, little is known about the role of individual HDACs in tumor cells or in their genesis. In accordance with the present invention, a unique HDAC isoform, HDAC9c, has been identified and characterized. HDAC9 shows restricted expression in normal adult tissues, but is overexpressed in several primary human tumors, including those derived from breast and prostate cancers. The overexpression of HDAC9c in *in vitro* models promoted the oncogenic transformation of fibroblasts and this transformed phenotype was associated with the induction of actin cytoskeletal stress fiber formation. These results suggest a functional consequence of HDAC9c overexpression is the promotion and/or maintenance of the transformation state of certain tumor cells.

Members of the HDAC protein family have been shown to possess potent ability to repress transcription. For instance, tumor suppressor genes p21 and gelsolin are expressed upon HDAC inhibition (Sowa et al., 1999, *Cancer Res.* 59(17):4266-70; Saito et al., 1999, *Proc. Natl. Acad. Sci. USA* 96:4592-4597). It is interesting to note that gelsolin negatively regulates the formation of the actin cytoskeleton (Sun et al., 1999, *J. Biol. Chem.* 274:33179-33182). In contrast, actin cytoskeleton formation is positively regulated by HDAC9c expression (FIG. 19). Thus, HDAC9c inhibition or overexpression may regulate gelsolin levels, and this regulation may underlie the cytoskeletal changes mediated by HDAC9c.

HDAC9 was overexpressed greater than 90% of the breast and prostate tumor specimens examined compared to corresponding tissue from normal patients (FIGS. 17A-17B). By comparison, the epidermal growth factor (EGF) receptor, erbB2, has been estimated to be overexpressed in roughly 30% of certain tumor types (King et al., 1985, *Science* 229:974-976). These observations strongly suggest that HDAC9c can be used as a diagnostic marker for breast or prostate tumorigenesis. Hormonal signaling is critical to the progression and treatment of breast cancers, and HDAC9 has been implicated in transcription (Zhou et al., *Proc. Natl. Acad. Sci. USA* 98:10572-10577). Without wishing to be bound by theory, it is possible that HDAC9 regulates estrogen or androgen responsive promoters in these tumor cells. As shown herein, HDAC9 expression is increased in primary cancers, and restricted in normal tissue expression. Further, HDAC9c expression induces oncogenic transformation. The sum of these observations indicates that HDAC9c can be used as a diagnostic and/or therapeutic target for certain tumors or cancers, in particular, breast and prostate tumors or cancers.

**EXAMPLE 14: HDAC9 SPLICE VARIANTS**

Using the methods described herein, HDAC9 splice variants were identified, including BMY_HDACX variant 1 (FIGS. 20A-20C; SEQ ID NO:94; also called BMY_HDACX_v1 and HDACX_v1) and BMY_HDACX variant 2 (FIGS. 21A-21B; SEQ ID NO:96; also called BMY_HDACX_v2 and HDACX_v2). The cDNA sequences for BMY_HDACX_v1 (SEQ ID NO:94) and BMY_HDACX_v2 (SEQ ID NO:96) were aligned to the nucleotide sequences of three reported splice products of the HDAC9 gene, including HDAC9v1 (NCBI Ref. Seq. NM_058176; FIGS. 22A-22C; SEQ ID NO:97), HDAC9v2 (NCBI Ref. Seq. NM_058177; FIGS. 22D-22F; SEQ ID NO:98), and HDAC9v3 (NCBI Ref. Seq. NM_014707; FIGS. 22G-22I; SEQ ID NO:100). The sequence alignment produced by ClustalW (D.G. Higgins et al., 1996, *Methods Enzymol.* 266:383-402) is shown in FIGS. 23A-23K.

ClustalW sequence alignments indicated that the HDAC9c amino acid sequence showed 80.5% identity to the HDAC9a (AY032738) amino acid sequence, 94.1% identity to the HDAC9 (AY032737) amino acid sequence,

and 55.1% identity to the HDAC5 (AF132608) amino acid sequence. The HDAC9c nucleotide sequence showed 81.4% identity to the HDAC9a (AY032738) nucleotide sequence, 94.3% identity to the HDAC9 (AY032737) nucleotide sequence, and 60.1% identity to the HDAC5 (AF132608) nucleotide sequence. In addition, the HDACX_v2 amino acid sequence showed 55.2% identity to the most closely related amino acid sequence, and the HDACX_v2 nucleotide sequence showed 55.3% identity to the HDAC9a (AY032738) nucleotide sequence, 48.1% identity to the HDAC9 (AY032737) nucleotide sequence, and 27.6% identity to the HDAC5 (AF132608) nucleotide sequence.

Additional amino acid sequence alignments are shown in FIGS. 24A-24D and FIGS. 25A-25C. For reference, the SEQ ID NOs of the sequences of the present invention are listed in the table shown below. HDACX_v1 and HDACX_v2 constructs were deposited at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 on _____ under ATCC Accession No. _____ _____ according to the terms of the Budapest Treaty.

| Description | SEQ ID NO: |
|---|---|
| BMY_HDAL1 nucleic acid sequence | SEQ ID NO:1 |
| BMY_HDAL1 amino acid sequence | SEQ ID NO:2 |
| BMY_HDAL1 reverse nucleic acid sequence | SEQ ID NO:3 |
| BMY_HDAL2 amino acid sequence | SEQ ID NO:4 |
| BMY_HDAL3 amino acid sequence | SEQ ID NO:5 |
| SC_HDA1 amino acid sequence | SEQ ID NO:6 |
| Human HDAC4 amino acid sequence | SEQ ID NO:7 |
| Human HDAC5 amino acid sequence | SEQ ID NO:8 |
| Human HDAC7 amino acid sequence | SEQ ID NO:9 |
| *Aquifex* ACUC HDAL amino acid sequence | SEQ ID NO:10 |
| AC002088 nucleic acid sequence | SEQ ID NO:11 |
| BMY_HDAL2 nucleic acid sequence | SEQ ID NO:12 |
| BMY_HDAL2 reverse nucleic acid sequence | SEQ ID NO:13 |
| AC002410 nucleic acid sequence | SEQ ID NO:14 |

| Description | SEQ ID NO: |
|---|---|
| N-terminus of BMY_HDAL3 | SEQ ID NO:15 |
| C-terminus of BMY_HDAL3 | SEQ ID NO:16 |
| BAC AC004994 nucleic acid sequence | SEQ ID NO:17 |
| BAC AC004744 nucleic acid sequence | SEQ ID NO:18 |
| BMY_HDAL3 nucleic acid sequence | SEQ ID NO:19 |
| BMY_HDAL3 reverse strand nucleic acid sequence | SEQ ID NO:20 |
| AAC78618 amino acid sequence | SEQ ID NO:21 |
| AAD15364 amino acid sequence | SEQ ID NO:22 |
| AA287983 nucleic acid sequence | SEQ ID NO:23 |
| BMY_HDAL1 single exon primer | SEQ ID NO:24 |
| BMY_HDAL1 single exon primer | SEQ ID NO:25 |
| BMY_HDAL1 single exon primer | SEQ ID NO:26 |
| BMY_HDAL1 single exon primer | SEQ ID NO:27 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:28 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:29 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:30 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:31 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:32 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:33 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:34 |
| BMY_HDAL1 multiple exon primer | SEQ ID NO:35 |
| BMY_HDAL1 capture oligonucleotide | SEQ ID NO:36 |
| BMY_HDAL1 5' oligo primer | SEQ ID NO:37 |
| BMY_HDAL1 3' oligo primer | SEQ ID NO:38 |
| BMY_HDAL2 single exon primer | SEQ ID NO:39 |
| BMY_HDAL2 single exon primer | SEQ ID NO:40 |
| BMY_HDAL2 single exon primer | SEQ ID NO:41 |
| BMY_HDAL2 single exon primer | SEQ ID NO:42 |
| BMY_HDAL2 single exon primer | SEQ ID NO:43 |
| BMY_HDAL2 single exon primer | SEQ ID NO:44 |
| BMY_HDAL2 single exon primer | SEQ ID NO:45 |
| BMY_HDAL2 single exon primer | SEQ ID NO:46 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:47 |

| Description | SEQ ID NO: |
|---|---|
| BMY_HDAL2 multiple exon primer | SEQ ID NO:48 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:49 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:50 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:51 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:52 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:53 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:54 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:55 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:56 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:57 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:58 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:59 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:60 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:61 |
| BMY_HDAL2 multiple exon primer | SEQ ID NO:62 |
| BMY_HDAL2 capture oligonucleotide | SEQ ID NO:63 |
| BMY_HDAL2 capture oligonucleotide | SEQ ID NO:64 |
| BMY_HDAL2 5' oligo primer | SEQ ID NO:65 |
| BMY_HDAL2 3' oligo primer | SEQ ID NO:66 |
| BMY_HDAL3 single exon primer | SEQ ID NO:67 |
| BMY_HDAL3 single exon primer | SEQ ID NO:68 |
| BMY_HDAL3 single exon primer | SEQ ID NO:69 |
| BMY_HDAL3 single exon primer | SEQ ID NO:70 |
| BMY_HDAL3 single exon primer | SEQ ID NO:71 |
| BMY_HDAL3 single exon primer | SEQ ID NO:72 |
| BMY_HDAL3 single exon primer | SEQ ID NO:73 |
| BMY_HDAL3 single exon primer | SEQ ID NO:74 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:75 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:76 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:77 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:78 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:79 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:80 |

| Description | SEQ ID NO: |
|---|---|
| BMY_HDAL3 multiple exon primer | SEQ ID NO:81 |
| BMY_HDAL3 multiple exon primer | SEQ ID NO:82 |
| BMY_HDAL3 capture oligo | SEQ ID NO:83 |
| BMY_HDAL3 capture oligo | SEQ ID NO:84 |
| BMY_HDAL3 capture oligo | SEQ ID NO:85 |
| BMY_HDAL3 capture oligo | SEQ ID NO:86 |
| HDAC9c amino acid sequence | SEQ ID NO:87 |
| HDAC9c nucleotide sequence | SEQ ID NO:88 |
| HDAC9 (AY032737) amino acid sequence | SEQ ID NO:89 |
| HDAC9a (AY032738) amino acid sequence | SEQ ID NO:90 |
| HDAC4 (ALF132608) amino acid sequence | SEQ ID NO:91 |
| HDAC9 probe | SEQ ID NO:92 |
| BMY_HDACX_v1 amino acid sequence | SEQ ID NO:93 |
| BMY_HDACX_v1 nucleotide sequence | SEQ ID NO:94 |
| BMY_HDACX_v2 amino acid sequence | SEQ ID NO:95 |
| BMY_HDACX_v2 nucleotide sequence | SEQ ID NO:96 |
| HDAC9v1 (NM_058176) amino acid sequence | SEQ ID NO:89 |
| HDAC9v1 (NM_058176) nucleotide sequence | SEQ ID NO:97 |
| HDAC9v2 (NM_058177) amino acid sequence | SEQ ID NO:90 |
| HDAC9v2 (NM_058177) nucleotide sequence | SEQ ID NO:98 |
| HDAC9v3 (NM_014707) amino acid sequence | SEQ ID NO:99 |
| HDAC9v3 (NM_014707) nucleotide sequence | SEQ ID NO:100 |
| HDAL1 primer | SEQ ID NO:101 |
| HDAL2 primer | SEQ ID NO:102 |
| HDAL3 primer | SEQ ID NO:103 |
| HDAC9 forward primer | SEQ ID NO:104 |
| HDAC9 reverse primer | SEQ ID NO:105 |
| HDAC consensus nucleotide sequence | SEQ ID NO:106 |
| HDAC consensus amino acid sequence | SEQ ID NO:107 |

The contents of all patents, patent applications, published PCT applications and articles, books, references, reference manuals and abstracts

cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the invention pertains.

As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present invention, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present invention. Many modifications and variations of the present invention are possible in light of the above teachings.

**WHAT IS CLAIMED IS:**

1. An isolated polynucleotide encoding a histone deacetylase polypeptide which consists of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95.

2. An isolated polynucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:19, SEQ ID NO:88, SEQ ID NO:94, and SEQ ID NO:96.

3. An primer consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO:24-27, SEQ ID NO:28-35, SEQ ID NO:39-46, SEQ ID NO:47-62, SEQ ID NO:65-66, SEQ ID NO:67-74, SEQ ID NO:75-82, and SEQ ID NO:104-105.

4. A probe consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:63-64, SEQ ID NO:83-86, SEQ ID NO92, and SEQ ID NO:101-103.

5. A cell line comprising the isolated polynucleotide according to claim 1.

6. An expression vector comprising the isolated polynucleotide according to claim 1.

7. A host cell comprising the expression vector according to claim 6, wherein the host cell is selected from the group consisting of bacterial, yeast, insect, mammalian, and human cells.

8. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95.

9. An antibody which binds specifically to the isolated polypeptide according to claim 8, wherein the antibody is selected from the group consisting of polyclonal and monoclonal antibodies.

10. An antisense polynucleotide which consists of a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:63-64, and SEQ ID NO:83-86.

11. An expression vector comprising the antisense polynucleotide according to claim 10.

12. A pharmaceutical composition selected from the group consisting of:

a. a pharmaceutical composition comprising a monoclonal antibody that specifically binds to an isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95, and a physiologically acceptable carrier, diluent, or excipient;

b. a pharmaceutical composition comprising an antisense polynucleotide which consists of a nucleotide sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:63-64, and SEQ ID NO:83-86, and a physiologically acceptable carrier, diluent, or excipient; and

c. a pharmaceutical composition comprising an expression vector comprising an isolated polynucleotide encoding a histone deacetylase polypeptide which consists of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:87, SEQ ID NO:93, and SEQ ID NO:95, and a physiologically acceptable carrier, diluent, or excipient.

13. A method of treating a cancer selected from the group consisting of breast and prostate cancer comprising administering the pharmaceutical composition according to claim 12 in an amount effective for treating the cancer.

14. A method of diagnosing a cancer selected from the group consisting of breast and prostate cancer comprising:

a. incubating the primer according to claim 3 with a biological sample under conditions to allow the primer to amplify a polynucleotide in the sample to produce a amplification product; and

b. measuring levels of amplification product formed in (a), wherein an alteration in these levels compared to standard levels indicates diagnosis of the cancer.

15. A method of diagnosing a cancer selected from the group consisting of breast and prostate cancer comprising:

a. incubating the probe according to claim 4 with a biological sample under conditions to allow the probe to hybridize with a polynucleotide in the sample to form a complex; and b.

measuring levels of hybridization complex formed in (a), wherein an alteration in these levels compared to standard levels indicates diagnosis of the cancer.

16. A method of diagnosing a cancer selected from the group consisting of breast and prostate cancer comprising:

a. contacting the antibody according to claim 9 with a biological sample under conditions to allow the antibody to associate with a polypeptide in the sample to form a complex; and

b. measuring levels of complex formed in (a), wherein an alteration in these levels compared to standard levels indicates diagnosis of the cancer.

17. A method of detecting a histone deacetylase polynucleotide comprising:

a. incubating the probe according to claim 4 with a biological sample under conditions to allow the probe to hybridize with a polynucleotide in the sample to form a complex; and b.

identifying the complex formed in (a), wherein identification of the complex indicates detection of a histone deacetylase polynucleotide.

18. A method of detecting a histone deacetylase polypeptide comprising:

a. incubating the antibody according to claim 9 with a biological sample under conditions to allow the antibody to associate with a polypeptide in the sample to form a complex; and

b. identifying the complex formed in (a), wherein identification of the complex indicates detection of a histone deacetylase polypeptide.

19. A method of screening test agents to identify a candidate bioactive agent comprising:

a. contacting the isolated polynucleotide according to claim 1 with test agents under conditions to allow a test agent to associate with the polynucleotide to form a complex; b.

detecting the complex of (b), wherein detection of the complex indicates identification of a candidate bioactive agent.

20. A method of screening test agents to identify a candidate bioactive agent comprising:

a. contacting the isolated polypeptide according to claim 8 with test agents under conditions to allow a test agent to associate with the polypeptide to form a complex;

b. detecting the complex of (b), wherein detection of the complex indicates identification a candidate bioactive agent.

```
   GlyIleAlaTyrAspProLeuMetLeuLysHisGlnCysValCysGly
1  ggaattgcctatgacccctgatgctgaaacaccagtgcgtttgtggc
   ccttaacggatactggggaactacgactttgtggtcacgcaaacaccg

   AsnSerThrThrHisProGluHisAlaGlyArgIleGlnSerIleTrp
49 aattccaccacccaccctgagcatgctggacgaatacagagtatctgg
   ttaaggtggtgggtgggactcgtacgacctgcttatgtctcatagacc

   SerArgLeuGlnGluThrGlyLeuLeuAsnLysCysGluArgIleGln
97 tcacgactgcaagaaactgggctgctaaataaatgtgagcgaattcaa
   agtgctgacgttctttgacccgacgatttatttacactcgcttaagtt

    GlyArgLysAlaSerLeuGluGluIleGlnLeuValHisSerGluHis
145 ggtcgaaaagccagcctggaggaaatacagcttgttcattctgaacat
    ccagcttttcggtcggacctcctttatgtcgaacaagtaagacttgta

    HisSerLeuLeuTyrGlyThrAsnProLeuAspGlyGlnLysLeuAsp
193 cactcactgttgtatggcaccaaccccctggacggacagaagctggac
    gtgagtgacaacataccgtggttgggggacctgcctgtcttcgacctg

    ProArgIleLeuLeuGlyAspAspSerGlnLysPhePheSerSerLeu
241 cccaggatactcctaggtgatgactctcaaaagtttttttcctcatta
    gggtcctatgaggatccactactgagagttttcaaaaaaaggagtaat

    ProCysGlyGlyLeuGlyValSerThr
289 ccttgtggtggacttggggtaagtaca
    ggaacaccacctgaaccccattcatgt
```

FIG. 1

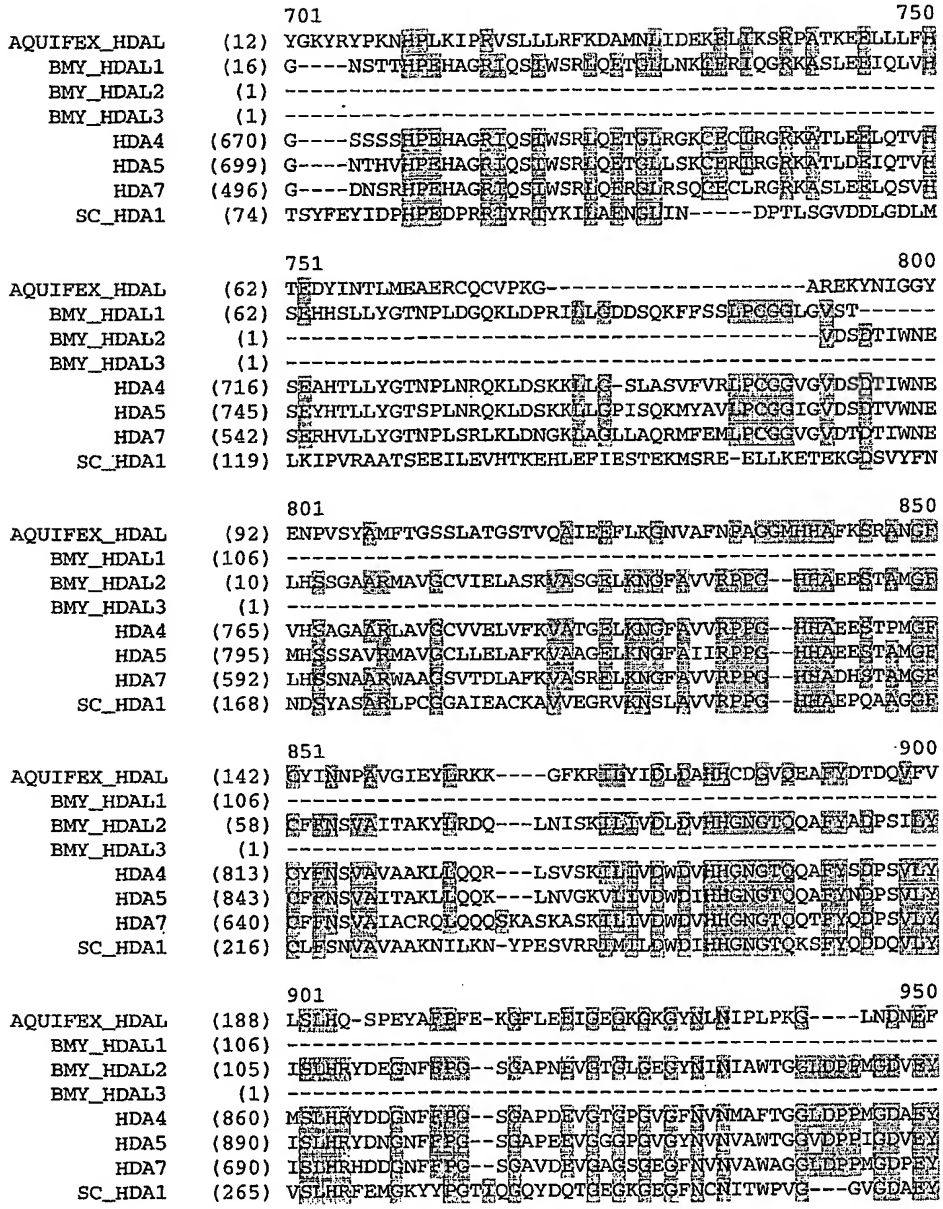



FIG. 2A

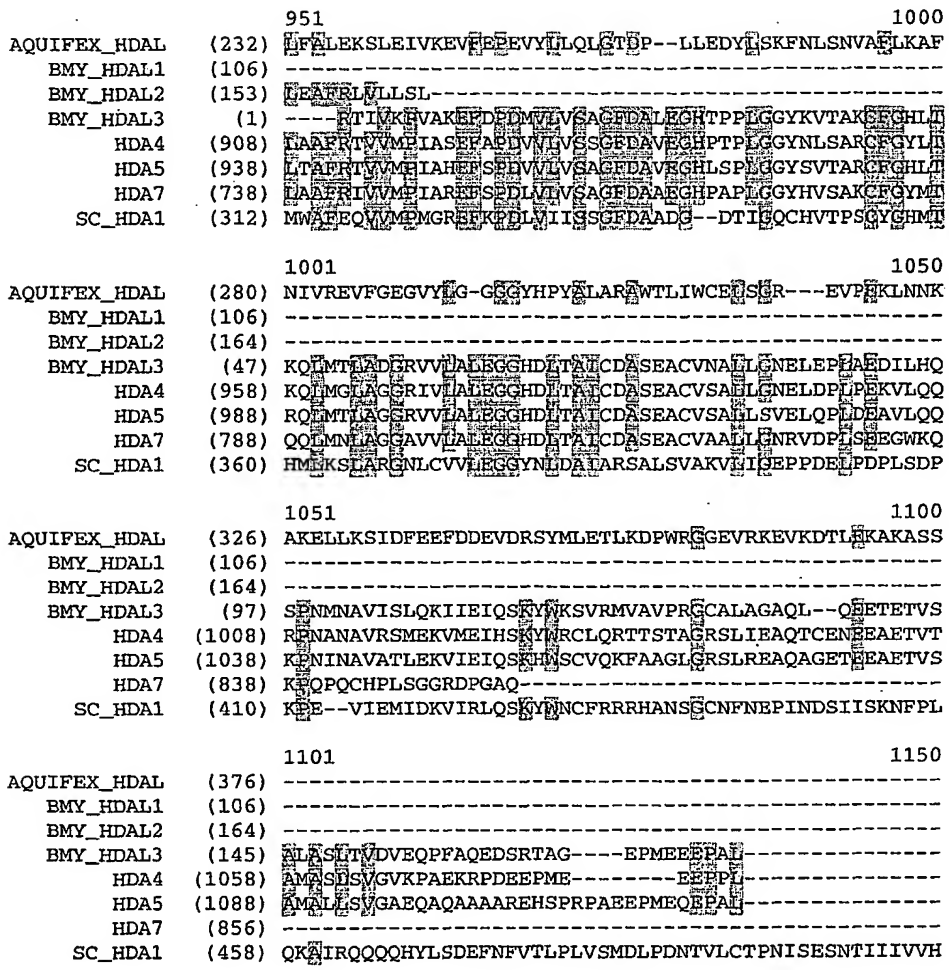

951 1000
AQUIFEX_HDAL (232) LFALEKSLEIVKEVFEPEVYLLQLGTDP--LLEDYLSKFNLSNVAFLKAF
BMY_HDAL1 (106) ----------
BMY_HDAL2 (153) LEAFRLVLLSL----------
BMY_HDAL3 (1) ----RTIVKRVAKEPDPDMVLVSACFDALEGHTPPLGGYKVTAKEFGHLL
HDA4 (908) LAAFRTVVMPIASEFAPLVVLVSSGFDAVEGHPTPLGGYNLSARCFGYLT
HDA5 (938) LTAFRTVVMPIAHEFSPDVVLVSAGFDAVEGHLSPLGGYSVTARCFGHLT
HDA7 (738) LAAFRIVVMPIARELSPDLVLVSAGFDAAEGHPAPLGGYHVSAKCFGYMT
SC_HDA1 (312) MWAFEQVVMPMGREFKPDLVIISSGFDAADG--DTIGQCHVTPSGYGHMT
1001 1050
AQUIFEX_HDAL (280) NIVREVFGEGVYLG-GGGYHPYALARAWTLIWCELSGR---EVPFKLNNK
BMY_HDAL1 (106) ----------
BMY_HDAL2 (164) ----------
BMY_HDAL3 (47) KQLMTLADGRVVLALEGGHDLTALCDASEACVNALLGNELEPLAEDILHQ
HDA4 (958) KQLMGLAGGRIVLALEGGHDLTAICDASEACVSALLGNELDPLPEKVLQQ
HDA5 (988) RQLMTLAGGRVVLALEGGHDLTAICDASEACVSALLSVELQPLDEAVLQQ
HDA7 (788) QQLMNLAGGAVVLALEGGHDLTALCDASEACVAALLGNRVDPLSEEGWKQ
SC_HDA1 (360) HMLKSLARGNLCVVLEGGYNLDAIARSALSVAKVLIGEPPDELPDPLSDP
1051 1100
AQUIFEX_HDAL (326) AKELLKSIDFEEFDDEVDRSYMLETLKDPWRGGEVRKEVKDTLFKAKASS
BMY_HDAL1 (106) ----------
BMY_HDAL2 (164) ----------
BMY_HDAL3 (97) SFNMNAVISLQKIIEIQSKYWKSVRMVAVPRGCALAGAQL--QFETETVS
HDA4 (1008) RFNANAVRSMEKVMEIHSKYWRCLQRTTSTAGRSLIEAQTCENEEAETVT
HDA5 (1038) KFNINAVATLEKVIEIQSKHWSCVQKFAAGLGRSLREAQAGETEEAETVS
HDA7 (838) KFQPQCHPLSGGRDPGAQ----------
SC_HDA1 (410) KFE--VIEMIDKVIRLQSKYWNCFRRRHANSGCNFNEPINDSIISKNFPL
1101 1150
AQUIFEX_HDAL (376) ----------
BMY_HDAL1 (106) ----------
BMY_HDAL2 (164) ----------
BMY_HDAL3 (145) ALASLTVDVEQPFAQEDSRTAG----EPMEEEPAL----------
HDA4 (1058) AMASDSVGVKPAEKRPDEEPME--------EEPPL----------
HDA5 (1088) AMALLSVGAEQAQAAAAREHSPRPAEEPMEQEPAL----------
HDA7 (856) ----------
SC_HDA1 (458) QKAIRQQQQHYLSDEFNFVTLPLVSMDLPDNTVLCTPNISESNTIIIVVH

FIG. 2B

```
Genewise results from HDA5_HUMAN_run2 applied to AC002088
Hit 1: bits = 149
      BAC start:56543
      BAC end:74703
      Protein start:684
      Protein end:788

>Results for GCGPROT:HDA5_HUMAN vs AC002088 (forward) [0]

genewisedb output
Score 149.09 bits over entire alignment.
This will be different from per-alignment scores. See manual for details
For computer parsable output, try genewisedb -help or read the manual
Scores as bits over a synchronous coding model

                    Alignment 1 Score 148.82 (Bits)


HDA5       684 G  V  V  Y  D  T  F  M  L  K  H  Q  C  M  C  G  N  T  H  V
               G  +     Y  D     +  M  L  K  H  Q  C  +  C  G  N  +
               G  I  A  Y  D  P  L  M  L  K  H  Q  C  V  C  G  N  S  T  T
AC002088 56543 ggaattgcctatgacccctgatgctgaaacaccagtgcgtttgtggcaattccaccacc

               H  P  E  H  A  G  R  I  Q  S  I  W  S  R  L  Q  E  T  G
               H  P  E  H  A  G  R  I  Q  S  I  W  S  R  L  Q  E  T  G
               H  P  E  H  A  G  R  I  Q  S  I  W  S  R  L  Q  E  T  G
               caccctgagcatgctggacgaatacagagtatctggtcacgactgcaagaaactggg

HDA5       723 L  L  S  K  C  E                          R  I  R  G  R  K
               L  L  +  K  C  E                          R  I  +  G  R  K
               L  L  N  K  C  E                          R  I  Q  G  R  K
AC002088 56660 ctgctaaataaatgtgagGTAATCC  Intron 1   CAGcgaattcaaggtcgaaaa
                                      <0-----[56678:69695]-0>

               A  T  L  D
               A  +  L  +
               A  S  L  E
               gccagcctggag

HDA5       739 E  I  Q  T  V  H  S  E  Y  H  T  L  L  Y  G  T  S  P  L  N
               E  I  Q     V  H  S  E  +  H  +  L  L  Y  G  T  +  P  L  +
               E  I  Q  L  V  H  S  E  H  H  S  L  L  Y  G  T  N  P  L  D
AC002088 69726 gaaatacagcttgttcattctgaacatcactcactgttgtatggcaccaacccctggac

               R  Q  K  L  D  S  K  K  L  L
                  Q  K  L  D     +     L  L
               G  Q  K  L  D  P  R  I  L  L
               ggacagaagctggaccccaggatactccta

HDA5  769                                  P  I  S  Q  K  M  Y  A  V  L  P
                                              S  Q  K  +  +  +     L  P
                           G:G[ggt]        D  D  S  Q  K  F  F  S  S  L  P
AC002088 69816 GGTCTGTA  Intron 2   TAGGTgatgactctcaaaagttttttttcctcattacct
                     <1-----[69817:74644]-1>
```

FIG. 3A

```
                    C  G  G  I  G  V  D  S
                    C  G  G  +  G  V     +
                    C  G  G  L  G  V  S  T
                    tgtggtggacttggggtaagtaca

HDA5        783 G  I  G  V  D  S
                G  +  G  V     +
                G  L  G  V  S  T
AC002088 74686 ggacttggggtaagtaca
```

FIG. 3B

```
MOTIFS FROM: BMY_HDAL1.AA.FASTA

 MISMATCHES: 0


  BMY_HDAL1.AA.FASTA  CHECK: 4620  LENGTH: 105    !

AMIDATION              XG(R,K)(R,K)
                         XG(R)(K)
            48: KCERI     QGRK      ASLEE

 (ABSTRACT FILE: 0009.PDOC)

ASN_GLYCOSYLATION     N~(P)(S,T)~(P)
                        N~P(T)~P
            17: QCVCG      NSTT       HPEHA

 (ABSTRACT FILE: 0001.PDOC)

CAMP_PHOSPHO_SITE     (R,K)2X(S,T)
                      (R,K){2}X(S)
            50: ERIQG      RKAS      LEEIQ

 (ABSTRACT FILE: 0004.PDOC)

CK2_PHOSPHO_SITE      (S,T)X2(D,E)
                       (T)X{2}(E)
            20: CGNST      THPE      HAGRI

                       (S)X{2}(E)
            53: QGRKA      SLEE      IQLVH

 (ABSTRACT FILE: 0006.PDOC)

MYRISTYL              G~(E,D,R,K,H,P,F,Y,W)X2(S,T,A,G,C,N)~(P)
                        G~(E,D,R,K,H,P,F,Y,W)X{2}(T)~P
            16: HQCVC                  GNSTTH                  PEHAG

                      G~(E,D,R,K,H,P,F,Y,W)X{2}(S)~P
           100: SLPCG                  GLGVST

 (ABSTRACT FILE: 0008.PDOC)

PKC_PHOSPHO_SITE      (S,T)X(R,K)
                        (S)X(K)
            89: LLGDD      SQK      FFSSL

 (ABSTRACT FILE: 0005.PDOC)
```

FIG. 4

```
      ValAspSerAspThrIleTrpAsnGluLeuHisSerSerGlyAlaAlaArgMetAlaVal
1     GTGGACAGTGACACCATTTGGAATGAGCTACACTCGTCCGGTGCTGCACGCATGGCTGTT
      CACCTGTCACTGTGGTAAACCTTACTCGATGTGAGCAGGCCACGACGTGCGTACCGACAA

      GlyCysValIleGluLeuAlaSerLysValAlaSerGlyGluLeuLysAsnGlyPheAla
61    GGCTGTGTCATCGAGCTGGCTTCCAAAGTGGCCTCAGGAGAGCTGAAGAATGGGTTTGCT
      CCGACACAGTAGCTCGACCGAAGGTTTCACCGGAGTCCTCTCGACTTCTTACCCAAACGA

      ValValArgProProGlyHisHisAlaGluGluSerThrAlaMetGlyPheCysPhePhe
121   GTTGTGAGGCCCCCTGGCCATCACGCTGAAGAATCCACAGCCATGGGGTTCTGCTTTTTT
      CAACACTCCGGGGGACCGGTAGTGCGACTTCTTAGGTGTCGGTACCCCAAGACGAAAAAA

      AsnSerValAlaIleThrAlaLysTyrLeuArgAspGlnLeuAsnIleSerLysIleLeu
181   AATTCAGTTGCAATTACCGCCAAATACTTGAGAGACCAACTAAATATAAGCAAGATATTG
      TTAAGTCAACGTTAATGGCGGTTTATGAACTCTCTGGTTGATTTATATTCGTTCTATAAC

      IleValAspLeuAspValHisHisGlyAsnGlyThrGlnGlnAlaPheTyrAlaAspPro
241   ATTGTAGATCTGGATGTTCACCATGGAAACGGTACCCAGCAGGCCTTTTATGCTGACCCC
      TAACATCTAGACCTACAAGTGGTACCTTTGCCATGGGTCGTCCGGAAAATACGACTGGGG

      SerIleLeuTyrIleSerLeuHisArgTyrAspGluGlyAsnPhePheProGlySerGly
301   AGCATCCTGTACATTTCACTCCATCGCTATGATGAAGGGAACTTTTTCCCTGGCAGTGGA
      TCGTAGGACATGTAAAGTGAGGTAGCGATACTACTTCCCTTGAAAAAGGGACCGTCACCT

      AlaProAsnGluValGlyThrGlyLeuGlyGluGlyTyrAsnIleAsnIleAlaTrpThr
361   GCCCCAAATGAGGTTGGAACAGGCCTTGGAGAAGGGTACAATATAAATATTGCCTGGACA
      CGGGGTTTACTCCAACCTTGTCCGGAACCTCTTCCCATGTTATATTTATAACGGACCTGT

      GlyGlyLeuAspProProMetGlyAspValGluTyrLeuGluAlaPheArgLeuValLeu
421   GGTGGCCTTGATCCTCCCATGGGAGATGTTGAGTACCTTGAAGCATTCAGGTTGGTACTT
      CCACCGGAACTAGGAGGGTACCCTCTACAACTCATGGAACTTCGTAAGTCCAACCATGAA

      LeuSerLeu
481   CTTTCTCTC
      GAAAGAGAG
```

FIG. 5

```
GENEWISE RESULTS FROM HDA5_HUMAN_RUN3 APPLIED TO AC002410
HIT 1: BITS = 262
      BAC START:15451
      BAC END:58122
      PROTEIN START:786
      PROTEIN END:948

>RESULTS FOR GCGPROT:HDA5_HUMAN VS AC002410 (FORWARD) [0]

GENEWISEDB OUTPUT
SCORE 262.30 BITS OVER ENTIRE ALIGNMENT.
THIS WILL BE DIFFERENT FROM PER-ALIGNMENT SCORES. SEE MANUAL FOR DETAILS
FOR COMPUTER PARSABLE OUTPUT, TRY GENEWISEDB -HELP OR READ THE MANUAL
SCORES AS BITS OVER A SYNCHRONOUS CODING MODEL

                    ALIGNMENT 1 SCORE 261.25 (BITS)


HDA5        786 V  D  S  D  T  V  W  N  E  M  H  S  S  S  A  V  R  M  A  V  G  C  L
                V  D  S  D  T  +  W  N  E  +  H  S  S     A     R  M  A  V  G  C  +
                V  D  S  D  T  I  W  N  E  L  H  S  S  G  A  A  R  M  A  V  G  C  V
AC002410 15451 GTGGACAGTGACACCATTTGGAATGAGCTACACTCGTCCGGTGCTGCACGCATGGCTGTTGGCTGTGTC

               L  E  L  A  F  K  V  A  A  G  E  L  K
               +  E  L  A     K  V  A  +  G  E  L  K
               I  E  L  A  S  K  V  A  S  G  E  L  K
               ATCGAGCTGGCTTCCAAAGTGGCCTCAGGAGAGCTGAAG

HDA5  822                                 N  G  F  A  I  I  R  P  P  G  H  H  A  E  E  S
                                          N  G  F  A  +  +  R  P  P  G  H  H  A  E  E  S
                                          N  G  F  A  V  V  R  P  P  G  H  H  A  E  E  S
AC002410 15559 GTGAGGT  INTRON 1    CAGAATGGGTTTGCTGTTGTGAGGCCCCCTGGCCATCACGCTGAAGAATCC
                     <0-----[15559:51266]-0>

HDA5        838 T  A                                 G  F  C  F  F  N  S  V  A  I  T
                T  A                                 G  F  C  F  F  N  S  V  A  I  T
                T  A                M:M[ATG]         G  F  C  F  F  N  S  V  A  I  T
AC002410 51315 ACAGCCATGTAAGTA  INTRON 2    CAGGGGGTTCTGCTTTTTTAATTCAGTTGCAATTACC
                          <2-----[51323:51566]-2>

HDA5        852 A  K  L  L  Q  Q  K  L  N  V  G  K  V  L  I  V  D  W
                A  K     L  +     +  L  N  +     K  +  L  I  V  D
                A  K  Y  L  R  D  Q  L  N  I  S  K  I  L  I  V  D  L
AC002410 51601 GCCAAATACTTGAGAGACCAACTAAATATAAGCAAGATATTGATTGTAGATCTGGTATGTA  INTRON 3
                                                                    <0---[51655:57572]

HDA5         870    D  I  H  H  G  N  G  T  Q  Q  A  F  Y  N  D  P  S  V  L  Y  I  S  L
                    D  +  H  H  G  N  G  T  Q  Q  A  F  Y     D  P  S  +  L  Y  I  S  L
                    D  V  H  H  G  N  G  T  Q  Q  A  F  Y  A  D  P  S  I  L  Y  I  S  L
AC002410  57570 TAGGATGTTCACCATGGAAACGGTACCCAGCAGGCCTTTTATGCTGACCCCAGCATCCTGTACATTTCACTC
                -0>
                    H  R  Y  D  N  G  N  F  F  P  G  S  G
                    H  R  Y  D     G  N  F  F  P  G  S  G
                    H  R  Y  D  E  G  N  F  F  P  G  S  G
                    CATCGCTATGATGAAGGGAACTTTTTCCCTGGCAGTGGA

HDA5        906 A  P  E  E                          V  G  G  G  P  G  V  G  Y  N  V  N
                A  P     E                          V  G     G     G     G  Y  N  +  N
                A  P  N  E                          V  G  T  G  L  G  E  G  Y  N  I  N
AC002410 57681 GCCCCAAATGAGGTTCGGT  INTRON 4    CAGGTTGGAACAGGCCTTGGAGAAGGGTACAATATAAAT
                          <0-----[57693:58005]-0>
```

FIG. 6A

```
HDA5          922 V  A  W  T  G  G  V  D  P  P  I  G  D  V  E  Y  L  T  A  F  R  T  V  V
                  +  A  W  T  G  G  +  D  P  P  +  G  D  V  E  Y  L     A  F  R     V  +
                  I  A  W  T  G  G  L  D  P  P  M  G  D  V  E  Y  L  E  A  F  R  L  V  L
AC002410    58042 ATTGCCTGGACAGGTGGCCTTGATCCTCCCATGGGAGATGTTGAGTACCTTGAAGCATTCAGGTTGGTACTT

                  M  P  I
                  +     +
                  L  S  L
                  CTTTCTCTC
```

FIG. 6B

```
PROSITE motifs identified in the partial predicted amino acid sequence of
BMY_HDAL2.
MOTIFS FROM: BMY_HDAL2.AA.FASTA

 MISMATCHES: 0


  BMY_HDAL2.AA.FASTA  CHECK: 2381  LENGTH: 163    !

ASN_GLYCOSYLATION      N~(P)(S,T)~(P)
                          N~P(S)~P
             75: LRDQL      NISK      ILIVD

                          N~P(T)~P
             90: DVHHG      NGTQ      QAFYA

 (ABSTRACT FILE: 0001.PDOC)

MYRISTYL               G~(E,D,R,K,H,P,F,Y,W)X2(S,T,A,G,C,N)~(P)
                          G~(E,D,R,K,H,P,F,Y,W)X{2}(A)~P
             91: VHHGN                    GTQQAF                YADPS

                          G~(E,D,R,K,H,P,F,Y,W)X{2}(G)~P
            126: APNEV                    GTGLGE                GYNIN

                          G~(E,D,R,K,H,P,F,Y,W)X{2}(G)~P
            128: NEVGT                    GLGEGY                NINIA

 (ABSTRACT FILE: 0008.PDOC)

PKC_PHOSPHO_SITE       (S,T)X(R,K)
                          (T)X(K)
             66: NSVAI      TAK      YLRDQ

 (ABSTRACT FILE: 0005.PDOC)
```

FIG. 7

```
GENEWISE RESULTS FROM HDA5_HUMAN_RUN3 APPLIED TO AC004994
HIT 1: BITS = 176
      BAC START:79767
      BAC END:11
      PROTEIN START:942
      PROTEIN END:1055

>RESULTS FOR GCGPROT:HDA5_HUMAN VS AC004994 (REVERSE) [0]

GENEWISEDB OUTPUT
SCORE 176.62 BITS OVER ENTIRE ALIGNMENT.
THIS WILL BE DIFFERENT FROM PER-ALIGNMENT SCORES. SEE MANUAL FOR DETAILS
FOR COMPUTER PARSABLE OUTPUT, TRY GENEWISEDB -HELP OR READ THE MANUAL
SCORES AS BITS OVER A SYNCHRONOUS CODING MODEL

                    ALIGNMENT 1 SCORE 174.85 (BITS)


HDA5_HUMAN  942 R  T  V  V  M  P  I  A  H  E  F  S  P  D  V  V  L  V  S  A  G  F  D  A
                R  T  +  V     P  +  A     E  F     P  D  +  V  L  V  S  A  G  F  D  A
                R  T  I  V  K  P  V  A  K  E  F  D  P  D  M  V  L  V  S  A  G  F  D  A
AC004994 -79767 AGGACCATCGTGAAGCCTGTGGCCAAAGAGTTTGATCCAGACATGGTCTTAGTATCTGCTGGATTTGATGCA
                V  E  G  H  L  S  P  L  G  G  Y  S  V  T  A
                +  E  G  H        P  L  G  G  Y     V  T  A
                L  E  G  H  T  P  P  L  G  G  Y  K  V  T  A
                TTGGAAGGCCACACCCCTCCTCTAGGAGGGTACAAAGTGACGGCA

HDA5_HUMAN  981 R                              F  G  H  L  T  R  Q  L  M  T  L  A
                    +                          F  G  H  L  T  +  Q  L  M  T  L  A
                    K              C:C[TGT]    F  G  H  L  T  K  Q  L  M  T  L  A
AC004994 -79650 AAATGTAAGTA  INTRON 1   TAGGTTTTGGTCATTTGACGAAGCAATTGATGACATTGGCT
                      <1-----[79646:18435]-1>

HDA5_HUMAN  995 G  G  R  V  V  L  A  L  E  G  G  H  D  L  T  A  I  C  D  A  S  E  A  C
                G  R  V  V  L  A  L  E  G  G  H  D  L  T  A  I  C  D  A  S  E  A  C
                D  G  R  V  V  L  A  L  E  G  G  H  D  L  T  A  I  C  D  A  S  E  A  C
AC004994 -18396 GATGGACGTGTGGTGTTGGCTCTAGAAGGAGGACATGATCTCACAGCCATCTGTGATGCATCAGAAGCCTGT
                V  S  A  L  L  S  V  E
                V  +  A  L  L        E
                V  N  A  L  L  G  N  E
                GTAAATGCCCTTCTAGGAAATGAG

HDA5_HUMAN 1027                          L  Q  P  L  D  E  A  V  L  Q  Q  K  P  N  I  N
                                         L  +  P  L     E     +  L     Q     P  N  +  N
                                         L  E  P  L  A  E  D  I  L  H  Q  S  P  N  M  N
AC004994 -18300 GTAAAAA  INTRON 2   CAGCTGGAGCCACTTGCAGAAGATATTCTCCACCAAAGCCCGAATATGAAT
                <0-----[18300:   98]-0>

HDA5_HUMAN 1043 A  V  A  T  L  E  K  V  I  E  I  Q  S
                A  V     +  L  +  K  +  I  E  I  Q  S
                A  V  I  S  L  Q  K  I  I  E  I  Q  S
AC004994    -49 GCTGTTATTTCTTTACAGAAGATCATTGAAATTCAAAGT
```

FIG. 8A

```
GENEWISE RESULTS FROM HDA5_HUMAN_RUN3 APPLIED TO AC004744
HIT 1: BITS = 57
      BAC START:85491
      BAC END:43563
      PROTEIN START:1022
      PROTEIN END:1122

>RESULTS FOR GCGPROT:HDA5_HUMAN VS AC004744 (REVERSE) [0]

GENEWISEDB OUTPUT
SCORE 57.38 BITS OVER ENTIRE ALIGNMENT.
THIS WILL BE DIFFERENT FROM PER-ALIGNMENT SCORES. SEE MANUAL FOR DETAILS
FOR COMPUTER PARSABLE OUTPUT, TRY GENEWISEDB -HELP OR READ THE MANUAL
SCORES AS BITS OVER A SYNCHRONOUS CODING MODEL

                    ALIGNMENT 1 SCORE 55.39 (BITS)

HDA5 1022        L  L  S  V  E  L  Q  P  L  D  E  A  V  L  Q  Q  K  P  N
                 L  L     +  +  L  +  P  L     E     +  L     Q     P  N
                 L  L  F  L  Q  L  E  P  L  A  E  D  I  L  H  Q  S  P  N
AC004744 -85491  CTACTATTCTTGCAGCTGGAGCCACTTGCAGAAGATATTCTCCACCAAAGCCCGAAT

                 I  N  A  V  A  T  L  E  K  V  I  E  I  Q
                 +  N  A  V     +  L  +  K  +  I  E  I  Q
                 M  N  A  V  I  S  L  Q  K  I  I  E  I  Q
                 ATGAATGCTGTTATTTCTTTACAGAAGATCATTGAAATTCAA

HDA5 1055                                  K  H  W  S  C  V  Q  K  F  A  A  G  L
                                           K  +  W        V  +        A
                           S:S[AGC]        K  Y  W  K  S  V  R  M  V  A  V  P  R
AC004744 85392   AGTATGTC  INTRON 1   TAGGCAAGTATTGGAAGTCAGTAAGGATGGTGGCTGTGCCAAGG
                 <1-----[85391:63817]-1>

HDA5 1069        G  R  S  L  R  E  A  Q  A  GET  E  E  A  E  T  V  S  A  M
                 G     +  L        A  Q           E  E     E  T  V  S  A  +
                 G  C  A  L  A  G  A  Q  L  --Q  E  E  T  E  T  V  S  A  L
AC004744 -63775  GGCTGTGCTCTGGCTGGTGCTCAGTTG  CAAGAGGAGACAGAGACCGTTTCTGCCCTG

                 A  L  L  S  V  G  A  E  Q  A  Q  A  AAARE  H
                 A     L  +  V        E  Q        A
                 A  S  L  T  V  D  V  E  Q  P  F  A  ----Q  E
                 GCCTCCCTAACAGTGGATGTGGAACAGCCCTTTGCT     CAGGAA

HDA5 1108        S  P                                  P  A  E  E  P  M  E  Q  E  P  A  L
                                                          A     E  P  M  E  +  E  P  A  L
                 D  S                 R:R[AGA]           T  A  G  E  P  M  E  E  E  P  A  L
AC004744 -63676  GACAGCAGGTATGAA  INTRON 2   CAGAACTGCTGGTGAGCCTATGGAAGAGGAGCCAGCCTTG
                        <2-----[63668:43600]-2>
```

FIG. 8B

```
                          1                                                50
»    AC004744     (1)
»    AC004994     (1)  aggaccatcgtgaagcctgtggccaaagagtttgatccagacatggtct
     BMY_HDAL3    (1)  aggaccatcgtgaagcctgtggccaaagagtttgatccagacatggtct

                         51                                               100
»    AC004744     (1)
»    AC004994    (50) tagtatctgctggatttgatgcattggaaggccacacccctcctctagga
     BMY_HDAL3   (50) tagtatctgctggatttgatgcattggaaggccacacccctcctctagga

                        101                                               150
»    AC004744     (1)
»    AC004994   (100) gggtacaaagtgacggcaaaatgttttggtcatttgacgaagcaattgat
     BMY_HDAL3  (100) gggtacaaagtgacggcaaaatgttttggtcatttgacgaagcaattgat

                        151                                               200
»    AC004744     (1)
»    AC004994   (150) gacattggctgatggacgtgtggtgttggctctagaaggaggacatgatc
     BMY_HDAL3  (150) gacattggctgatggacgtgtggtgttggctctagaaggaggacatgatc

                        201                                               250
»    AC004744     (1)
»    AC004994   (200) tcacagccatctgtgatgcatcagaagcctgtgtaaatgcccttctagga
     BMY_HDAL3  (200) tcacagccatctgtgatgcatcagaagcctgtgtaaatgcccttctagga

                        251                                               300
»    AC004744     (1)     agctggagccacttgcagaagatattctccaccaaagcccgaatat
»    AC004994   (250) aatgagctggagccacttgcagaagatattctccaccaaagcccgaatat
     BMY_HDAL3  (250) aatgagctggagccacttgcagaagatattctccaccaaagcccgaatat

                        301                                               350
»    AC004744    (50) gaatgctgttatttctttacagaagatcattgaaattcaaagcaagtatt
»    AC004994   (300) gaatgctgttatttctttacagaagatcattgaaattcaaa
     BMY_HDAL3  (300) gaatgctgttatttctttacagaagatcattgaaattcaaagcaagtatt

                        351                                               400
»    AC004744   (100) ggaagtcagtaaggatggtggctgtgccaaggggctgtgctctggctggt
»    AC004994 (•340)
     BMY_HDAL3  (350) ggaagtcagtaaggatggtggctgtgccaaggggctgtgctctggctggt

                        401                                               450
»    AC004744   (150) gctcagttgcaagaggagacagagaccgtttctgccctggcctccctaac
»    AC004994 (•340)
     BMY_HDAL3  (400) gctcagttgcaagaggagacagagaccgtttctgccctggcctccctaac

                        451                                               500
»    AC004744   (200) agtggatgtggaacagccctttgctcaggaagacagcagaactgctggtg
»    AC004994 (•340)
     BMY_HDAL3  (450) agtggatgtggaacagccctttgctcaggaagacagcagaactgctggtg

                        501                  525
»    AC004744   (250) agcctatggaagaggagccagcctt
»    AC004994 (•340)
     BMY_HDAL3  (500) agcctatggaagaggagccagcctt
```

FIG. 9

```
      ArgThrIleValLysProValAlaLysGluPheAspProAspMetValLeuValSerAla
1     AGGACCATCGTGAAGCCTGTGGCCAAAGAGTTTGATCCAGACATGGTCTTAGTATCTGCT
      TCCTGGTAGCACTTCGGACACCGGTTTCTCAAACTAGGTCTGTACCAGAATCATAGACGA

      GlyPheAspAlaLeuGluGlyHisThrProProLeuGlyGlyTyrLysValThrAlaLys
61    GGATTTGATGCATTGGAAGGCCACACCCCTCCTCTAGGAGGGTACAAAGTGACGGCAAAA
      CCTAAACTACGTAACCTTCCGGTGTGGGGAGGAGATCCTCCCATGTTTCACTGCCGTTTT

      CysPheGlyHisLeuThrLysGlnLeuMetThrLeuAlaAspGlyArgValValLeuAla
121   TGTTTTGGTCATTTGACGAAGCAATTGATGACATTGGCTGATGGACGTGTGGTGTTGGCT
      ACAAAACCAGTAAACTGCTTCGTTAACTACTGTAACCGACTACCTGCACACCACAACCGA

      LeuGluGlyGlyHisAspLeuThrAlaIleCysAspAlaSerGluAlaCysValAsnAla
181   CTAGAAGGAGGACATGATCTCACAGCCATCTGTGATGCATCAGAAGCCTGTGTAAATGCC
      GATCTTCCTCCTGTACTAGAGTGTCGGTAGACACTACGTAGTCTTCGGACACATTTACGG

      LeuLeuGlyAsnGluLeuGluProLeuAlaGluAspIleLeuHisGlnSerProAsnMet
241   CTTCTAGGAAATGAGCTGGAGCCACTTGCAGAAGATATTCTCCACCAAAGCCCGAATATG
      GAAGATCCTTTACTCGACCTCGGTGAACGTCTTCTATAAGAGGTGGTTTCGGGCTTATAC

      AsnAlaValIleSerLeuGlnLysIleIleGluIleGlnSerLysTyrTrpLysSerVal
301   AATGCTGTTATTTCTTTACAGAAGATCATTGAAATTCAAAGCAAGTATTGGAAGTCAGTA
      TTACGACAATAAAGAAATGTCTTCTAGTAACTTTAAGTTTCGTTCATAACCTTCAGTCAT

      ArgMetValAlaValProArgGlyCysAlaLeuAlaGlyAlaGlnLeuGlnGluGluThr
361   AGGATGGTGGCTGTGCCAAGGGGCTGTGCTCTGGCTGGTGCTCAGTTGCAAGAGGAGACA
      TCCTACCACCGACACGGTTCCCCGACACGAGACCGACCACGAGTCAACGTTCTCCTCTGT

      GluThrValSerAlaLeuAlaSerLeuThrValAspValGluGlnProPheAlaGlnGlu
421   GAGACCGTTTCTGCCCTGGCCTCCCTAACAGTGGATGTGGAACAGCCCTTTGCTCAGGAA
      CTCTGGCAAAGACGGGACCGGAGGGATTGTCACCTACACCTTGTCGGGAAACGAGTCCTT

      AspSerArgThrAlaGlyGluProMetGluGluGluProAlaLeu
481   GACAGCAGAACTGCTGGTGAGCCTATGGAAGAGGAGCCAGCCTTG
      CTGTCGTCTTGACGACCACTCGGATACCTTCTCCTCGGTCGGAAC
```

FIG. 10

```
PROSITE MOTIFS FROM: BMY_HDAL3.AA.FASTA

 MISMATCHES:0


  BMY_HDAL3.AA.FASTA  CHECK: 3930  LENGTH: 175    !

CK2_PHOSPHO_SITE       (S,T)X2(D,E)
                        (T)X{2}(D)
            51: TKQLM     TLAD     GRVVL

                       (T)X{2}(E)
           164: QEDSR     TAGE     PMEEE

 (ABSTRACT FILE: 0006.PDOC)

MYRISTYL               G~(E,D,R,K,H,P,F,Y,W)X2(S,T,A,G,C,N)~(P)
                            G~(E,D,R,K,H,P,F,Y,W)X{2}(A)~P
           128: VAVPR                  GCALAG                  AQLQE

 (ABSTRACT FILE: 0008.PDOC)

PKC_PHOSPHO_SITE       (S,T)X(R,K)
                         (T)X(K)
            38: GGYKV     TAK     CFGHL

                       (S)X(R)
           119: SKYWK     SVR     MVAVP

 (ABSTRACT FILE: 0005.PDOC)
```

FIG. 11

Multiple sequence alignment of BMY_HDAL3, AAC78618 and AAD15364

```
                    1                                                  50
AAC78618      (1)  -TTLVKPVAKEFDPDMVLVSAGFDALEGHTPPLGGYKVTAKCFGHLTKQLM
AAD15364      (1)  ------------------------------------------------------
BMY_HDAL3     (1)  RTTLVKPVAKEFDPDMVLVSAGFDALEGHTPPLGGYKVTAKCFGHLTKQLM

                    51                                                 100
AAC78618     (50)  TLADGRVVLALEGGHDLTAICDASEACVNALLGNELEPLAEDILHQSPNM
AAD15364      (1)  ------------------------------------LEPLAEDILHQSPNM
BMY_HDAL3    (51)  TLADGRVVLALEGGHDLTAICDASEACVNALLGNELEPLAEDILHQSPNM

                   101                                                 150
AAC78618     (100)  NAVISLQKIIEIQ-----------------------------------------
AAD15364      (16)  NAVISLQKIIEIQKLLVSLWKRSQPCEVPSPPLIFPVCDIIVYPPTPVPS
BMY_HDAL3    (101)  NAVISLQKIIEIQSKYWKSVRMVAVPRGCALAGAQLQEETETVSALASLT

                   151                 175
AAC78618     (113)  ---------------------
AAD15364      (66)  DMSCLLPGWHRFNGT-------
BMY_HDAL3    (151)  VDVEQPFAQEDSRTAGEPMEEEPAL
```

FIG. 12

BLASTN alignment of AA287983 and BMY_HDAL3

```
SCORE =  224 BITS (113), EXPECT = 4E-57
 IDENTITIES = 120/121 (99%), GAPS = 1/121 (0%)
 STRAND = PLUS / MINUS

BMY_HDAL3: 405 ATTTTGCCGTCACTTTGTACCCTCCTAGAGGAGGGGTGTGGCCTTCCAATGCATCAAATC
464
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AA287983:  207 ATTTTGCCGTCACTTTGTACCCTCCTAGAGGAGGGGTGTGGCCTTCCAATGCATCAAATC
148

BMY_HDAL3: 465 CAGCAGATACTAAGACCATGTCTGGATCAAACTCTTTGGCCACAGGCTTCACGATGGTCC
524
               |||||||||||||||||||||||||||||||| ||||||||||||||||||||||
AA287983:  147 CAGCAGATACTAAGACCATGTCTGGATCAAACTCTTT-GCCACAGGCTTCACGATGGTCC 89

BMY_HDAL3: 525 T 525
               |
AA287983:  88  T 88
```

FIG. 13

***Aquifex ACUC Protein***

```
  1 MKKVKLIGTL DYGKYRYPKN HPLKIPRVSL LLRFKDAMNL IDEKELIKSR
 51 PATKEELLLF HTEDYINTLM EAERCQCVPK GAREKYNIGG YENPVSYAMF
101 TGSSLATGST VQAIEEFLKG NVAFNPAGGM HHAFKSRANG FCYINNPAVG
151 IEYLRKKGFK RILYIDLDAH HCDGVQEAFY DTDQVFVLSL HQSPEYAFPF
201 EKGFLEEIGE GKGKGYNLNI PLPKGLNDNE FLFALEKSLE IVKEVFEPEV
251 YLLQLGTDPL LEDYLSKFNL SNVAFLKAFN IVREVFGEGV YLGGGGYHPY
301 ALARAWTLIW CELSGREVPE KLNNKAKELL KSIDFEEFDD EVDRSYMLET
351 LKDPWRGGEV RKEVKDTLEK AKASS
```

FIG. 14A

***Saccharomyces Cerevisiae Histone Deacetylase 1***

```
  1 MDSVMVKKEV LENPDHDLKR KLEENKEEEN SLSTTSKSKR QVIVPVCMPK
 51 IHYSPLKTGL CYDVRMRYHA KIFTSYFEYI DPHPEDPRRI YRIYKILAEN
101 GLINDPTLSG VDDLGDLMLK IPVRAATSEE ILEVHTKEHL EFIESTEKMS
151 REELLKETEK GDSVYFNNDS YASARLPCGG AIEACKAVVE GRVKNSLAVV
201 RPPGHHAEPQ AAGGFCLFSN VAVAAKNILK NYPESVRRIM ILDWDIHHGN
251 GTQKSFYQDD QVLYVSLHRF EMGKYYPGTI QGQYDQTGEG KGEGFNCNIT
301 WPVGGVGDAE YMWAFEQVVM PMGREFKPDL VIISSGFDAA DGDTIGQCHV
351 TPSCYGHMTH MLKSLARGNL CVVLEGGYNL DAIARSALSV AKVLIGEPPD
401 ELPDPLSDPK PEVIEMIDKV IRLQSKYWNC FRRRHANSGC NFNEPINDSI
451 ISKNFPLQKA IRQQQQHYLS DEFNFVTLPL VSMDLPDNTV LCTPNISESN
501 TIIIVVHDTS DIWAKRNVIS GTIDLSSSVI IDNSLDFIKW GLDRKYGIID
551 VNIPLTLFEP DNYSGMITSQ EVLIYLWDNY IKYFPSVAKI AFIGIGDSYS
601 GIVHLLGHRD TRAVTKTVIN FLGDKQLKPL VPLVDETLSE WYFKNSLIFS
651 NNSHQCWKEN ESRKPRKKFG RVLRCDTDGL NNIIEERFEE ATDFILDSFE
701 EWSDEE
```

FIG. 14B

***Homo Sapiens Histone Deacetylase 4***

```
   1 MSSQSHPDGL SGRDQPVELL NPARVNHMPS TVDVATALPL QVAPSAVPMD
  51 LRLDHQFSLP VAEPALREQQ LQQELLALKQ KQQIQRQILI AEFQRQHEQL
 101 SRQHEAQLHE HIKQQQEMLA MKHQQELLEH QRKLERHRQE QELEKQHREQ
 151 KLQQLKNKEK GKESAVASTE VKMKLQEFVL NKKKALAHRN LNHCISSDPR
 201 YWYGKTQHSS LDQSSPPQSG VSTSYNHPVL GMYDAKDDFP LRKTASEPNL
 251 KLRSRLKQKV AERRSSPLLR RKDGPVVTAL KKRPLDVTDS ACSSAPGSGP
 301 SSPNNSSGSV SAENGIAPAV PSIPAETSLA HRLVAREGSA APLPLYTSPS
 351 LPNITLGLPA TGPSAGTAGQ QDTERLTLPA LQQRLSLFPG THLTPYLSTS
 401 PLERDGGAAH SPLLQHMVLL EQPPAQAPLV TGLGALPLHA QSLVGADRVS
 451 PSIHKLRQHR PLGRTQSAPL PQNAQALQHL VIQQQHQQFL EKHKQQFQQQ
 501 QLQMNKIIPK PSEPARQPES HPEETEEELR EHQALLDEPY LDRLPGQKEA
 551 HAQAGVQVKQ EPIESDEEEA EPPREVEPGQ RQPSEQELLF RQQALLLEQQ
 601 RIHQLRNYQA SMEAAGIPVS FGGHRPLSRA QSSPASATFP VSVQEPPTKP
 651 RFTTGLVYDT LMLKHQCTCG SSSSHPEHAG RIQSIWSRLQ ETGLRGKCEC
 701 IRGRKATLEE LQTVHSEAHT LLYGTNPLNR QKLDSKKLLG SLASVFVRLP
 751 CGGVGVDSDT IWNEVHSAGA ARLAVGCVVE LVFKVATGEL KNGFAVVRPP
 801 GHHAEESTPM GFCYFNSVAV AAKLLQQRLS VSKILIVDWD VHHGNGTQQA
 851 FYSDPSVLYM SLHRYDDGNF FPGSGAPDEV GTGPGVGFNV NMAFTGGLDP
 901 PMGDAEYLAA FRTVVMPIAS EFAPDVVLVS SGFDAVEGHP TPLGGYNLSA
 951 RCFGYLTKQL MGLAGGRIVL ALEGGHDLTA ICDASEACVS ALLGNELDPL
1001 PEKVLQQRPN ANAVRSMEKV MEIHSKYWRC LQRTTSTAGR SLIEAQTCEN
1051 EEAETVTAMA SLSVGVKPAE KRPDEEPMEE EPPL
```

FIG. 14C

***Homo Sapiens Histone Deacetylase 5***

```
   1 MNSPNESDGM SGREPSLEIL PRTSLHSIPV TVEVKPVLPR AMPSSMGGGG
  51 GGSPSPVELR GALVGSVDPT LREQQLQQEL LALKQQQQLQ KQLLFAEFQK
 101 QHDHLTRQHE VQLQKHLKQQ QEMLAAKQQQ EMLAAKRQQE LEQQRQREQQ
 151 RQEELEKQRL EQQLLILRNK EKSKESAIAS TEVKLRLQEF LLSKSKEPTP
 201 GGLNHSLPQH PKCWGAHHAS LDQSSPPQSG PPGTPPSYKL PLPGPYDSRD
 251 DFPLRKTASE PNLKVRSRLK QKVAERRSSP LLRRKDGTVI STFKKRAVEI
 301 TGAGPGASSV CNSAPGSGPS SPNSSHSTIA ENGFTGSVPN IPTEMLPQHR
 351 ALPLDSSPNQ FSLYTSPSLP NISLGLQATV TVTNSHLTAS PKLSTQQEAE
 401 RQALQSLRQG GTLTGKFMST SSIPGCLLGV ALEGDGSPHG HASLLQHVLL
 451 LEQARQQSTL IAVPLHGQSP LVTGERVATS MRTVGKLPRH RPLSRTQSSP
 501 LPQSPQALQQ LVMQQQHQQF LEKQKQQQLQ LGKILTKTGE LPRQPTTHPE
 551 ETEEELTEQQ EVLLGEGALT MPREGSTESE STQEDLEEED EEEDGEEEED
 601 CIQVKDEEGE SGAEEGPDLE EPGAGYKKLF SDAQPLQPLQ VYQAPLSLAT
 651 VPHQALGRTQ SSPAAPGGMK SPPDQPVKHL FTTGVVYDTF MLKHQCMCGN
 701 THVHPEHAGR IQSIWSRLQE TGLLSKCERI RGRKATLDEI QTVHSEYHTL
 751 LYGTSPLNRQ KLDSKKLLGP ISQKMYAVLP CGGIGVDSDT VWNEMHSSSA
 801 VRMAVGCLLE LAFKVAAGEL KNGFAIIRPP GHHAEESTAM GFCFFNSVAI
 851 TAKLLQQKLN VGKVLIVDWD IHHGNGTQQA FYNDPSVLYI SLHRYDNGNF
 901 FPGSGAPEEV GGGPGVGYNV NVAWTGGVDP PIGDVEYLTA FRTVVMPIAH
 951 EFSPDVVLVS AGFDAVEGHL SPLGGYSVTA RCFGHLTRQL MTLAGGRVVL
1001 ALEGGHDLTA ICDASEACVS ALLSVELQPL DEAVLQQKPN INAVATLEKV
1051 IEIQSKHWSC VQKFAAGLGR SLREAQAGET EEAETVSAMA LLSVGAEQAQ
1101 AAAAREHSPR PAEEPMEQEP AL
```

FIG. 14D

***Homo Sapiens Histone Deacetylase 7***

```
  1 MDLRVGQRPP VEPPPEPTLL ALQRPQRLHH HLFLAGLQQQ RSVEPMRLSM
 51 DTPMPELQVG PQEQELRQLL HKDKSKRSAV ASSVVKQKLA EVILKKQQAA
101 LERTVHPNSP GIPYRTLEPL ETEGATRSML SSFLPPVPSL PSDPPEHFPL
151 RKTVSEPNLK LRYKPKKSLE RRKNPLLRKE SAPPSLRRRP AETLGDSSPS
201 SSSTPASGCS SPNDSEHGPN PILGDSDRRT HPTLGPRGPI LGSPHTPLFL
251 PHGLEPEAGG TLPSRLQPIL LLDPSGSHAP LLTVPGLGPL PFHFAQSLMT
301 TERLSGSGLH WPLSRTRSEP LPPSATAPPP PGPMQPRLEQ LKTHVQVIKR
351 SAKPSEKPRL RQIPSAEDLE TDGGGPGQVV DDGLEHRELG HGQPEARGPA
401 PLQQHPQVLL WEQQRLAGRL PRGSTGDTVL LPLAQGGHRP LSRAQSSPAA
451 PASLSAPEPA SQARVLSSSE TPARTLPFTT GLIYDSVMLK HQCSCGDNSR
501 HPEHAGRIQS IWSRLQERGL RSQCECLRGR KASLEELQSV HSERHVLLYG
551 TNPLSRLKLD NGKLAGLLAQ RMFEMLPCGG VGVDTDTIWN ELHSSNAARW
601 AAGSVTDLAF KVASRELKNG FAVVRPPGHH ADHSTAMGFC FFNSVAIACR
651 QLQQQSKASK ASKILIVDWD VHHGNGTQQT FYQDPSVLYI SLHRHDDGNF
701 FPGSGAVDEV GAGSGEGFNV NVAWAGGLDP PMGDPEYLAA FRIVVMPIAR
751 EFSPDLVLVS AGFDAAEGHP APLGGYHVSA KCFGYMTQQL MNLAGGAVVL
801 ALEGGHDLTA ICDASEACVA ALLGNRVDPL SEEGWKQKPQ PQCHPLSGGR
851 DPGAQ
```

FIG. 14E

***Human EST AA287983***

```
  1 ggccttggagaagggtacaatataaatattgcctggacaggtggcctt
 49 gatcctcccatgggagatgttgagtaccttgaagcattcaggaccatc
 97 gtgaagcctgtggcaaagagtttgatccagacatggtcttagtatctg
145 ctggatttgatgcattggaaggccacacccctcctctaggagggtaca
193 aagtgacggcaaaataaactcctgtgctggaggtacaacagtttggaa
241 gtatacttggggaaagagaaaacacaagatggaaggaagatctctctt
289 ttcacatcgggagcac
```

FIG. 14F

***Human predicted protein AAD15364***

```
  1 LEPLAEDILH QSPNMNAVIS LQKIIEIQKL LVSLWKRSQP CEVPSPPLIF
 51 PVCDIIVYPP TPVPSDMSCL LPGWHRFNGT
```

FIG. 14G

***Human predicted protein AAC78618***

```
  1 TIVKPVAKEF DPDMVLVSAG FDALEGHTPP LGGYKVTAKC FGHLTKQLMT
 51 LADGRVVLAL EGGHDLTAIC DASEACVNAL LGNELEPLAE DILHQSPNMN
101 AVISLQKIIE IQ
```

FIG. 14H

```
   1  ATGCACAGTATGATCAGCTCAGTGGATGTGAAGTCAGAAGTTCCTGTGGGCCTGGAGCCC   60
   1  M  H  S  M  I  S  S  V  D  V  K  S  E  V  P  V  G  L  E  P    20

  61  ATCTCACCTTTAGACCTAAGGACAGACCTCAGGATGATGATGCCCGTGGTGGACCCTGTT  120
  21  I  S  P  L  D  L  R  T  D  L  R  M  M  M  P  V  V  D  P  V   40

 121  GTCCGTGAGAAGCAATTGCAGCAGGAATTACTTCTTATCCAGCAGCAGCAACAAATCCAG  180
  41  V  R  E  K  Q  L  Q  Q  E  L  L  L  I  Q  Q  Q  Q  Q  I  Q   60

 181  AAGCAGCTTCTGATAGCAGAGTTTCAGAAACAGCATGAGAACTTGACACGGCAGCACCAG  240
  61  K  Q  L  L  I  A  E  F  Q  K  Q  H  E  N  L  T  R  Q  H  Q   80

 241  GCTCAGCTTCAGGAGCATATCAAGTTGCAACAGGAACTTCTAGCCATAAAACAGCAACAA  300
  81  A  Q  L  Q  E  H  I  K  L  Q  Q  E  L  L  A  I  K  Q  Q  Q  100

 301  GAACTCCTAGAAAAGGAGCAGAAACTGGAGCAGCAGAGGCAAGAACAGGAAGTAGAGAGG  360
 101  E  L  L  E  K  E  Q  K  L  E  Q  Q  R  Q  E  Q  E  V  E  R  120

 361  CATCGCAGAGAACAGCAGCTTCCTCCTCTCAGAGGCAAAGATAGAGGACGAGAAAGGGCA  420
 121  H  R  R  E  Q  Q  L  P  P  L  R  G  K  D  R  G  R  E  R  A  140

 421  GTGGCAAGTACAGAAGTAAAGCAGAAGCTTCAAGAGTTCCTACTGAGTAAATCAGCAACG  480
 141  V  A  S  T  E  V  K  Q  K  L  Q  E  F  L  L  S  K  S  A  T  160

 481  AAAGACACTCCAACTAATGGAAAAAATCATTCCGTGAGCCGCCATCCCAAGCTCTGGTAC  540
 161  K  D  T  P  T  N  G  K  N  H  S  V  S  R  H  P  K  L  W  Y  180

 541  ACGGCTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACATCTCCA  600
 181  T  A  A  H  H  T  S  L  D  Q  S  S  P  P  L  S  G  T  S  P  200

 601  TCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGATTTCCCCCTTCGAAAA  660
 201  S  Y  K  Y  T  L  P  G  A  Q  D  A  K  D  D  F  P  L  R  K  220

 661  ACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCAGGTTAAAACAGAAAGTGGCAGAGAGG  720
 221  T  A  S  E  P  N  L  K  V  R  S  R  L  K  Q  K  V  A  E  R  240

 721  AGAAGCAGCCCCTTACTCAGGCGGAAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGA  780
 241  R  S  S  P  L  L  R  R  K  D  G  N  V  V  T  S  F  K  K  R  260

 781  ATGTTTGAGGTGACAGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCA  840
 261  M  F  E  V  T  E  S  S  V  S  S  S  S  P  G  S  G  P  S  S  280

 841  CCAAACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCCCCTACC  900
 281  P  N  N  G  P  T  G  S  V  T  E  N  E  T  S  V  L  P  P  T  300

 901  CCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCATGAAGATTCCATGAAC  960
 301  P  H  A  E  Q  M  V  S  Q  Q  R  I  L  I  H  E  D  S  M  N  320

 961  CTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCAACATTACCTTGGGGCTTCCCGCAGTG 1020
 321  L  L  S  L  Y  T  S  P  S  L  P  N  I  T  L  G  L  P  A  V  340

1021  CCATCCCAGCTCAATGCTTCGAATTCACTCAAAGAAAAGCAGAAGTGTGAGACGCAGACG 1080
 341  P  S  Q  L  N  A  S  N  S  L  K  E  K  Q  K  C  E  T  Q  T  360

1081  CTTAGGCAAGGTGTTCCTCTGCCTGGGCAGTATGGAGGCAGCATCCCGGCATCTTCCAGC 1140
 361  L  R  Q  G  V  P  L  P  G  Q  Y  G  G  S  I  P  A  S  S  S  380

1141  CACCCTCATGTTACTTTAGAGGGAAAGCCACCCAACAGCAGCCACCAGGCTCTCCTGCAG 1200
 381  H  P  H  V  T  L  E  G  K  P  P  N  S  S  H  Q  A  L  L  Q  400

1201  CATTTATTATTGAAAGAACAAATGCGACAGCAAAAGCTTCTTGTAGCTGGTGGAGTTCCC 1260
 401  H  L  L  L  K  E  Q  M  R  Q  Q  K  L  L  V  A  G  G  V  P  420

1261  TTACATCCTCAGTCTCCCTTGGCAACAAAAGAGAGAATTTCACCTGGCATTAGAGGTACC 1320
 421  L  H  P  Q  S  P  L  A  T  K  E  R  I  S  P  G  I  R  G  T  440

1321  CACAAATTGCCCCGTCACAGACCCCTGAACCGAACCCAGTCTGCACCTTTGCCTCAGAGC 1380
 441  H  K  L  P  R  H  R  P  L  N  R  T  Q  S  A  P  L  P  Q  S  460

1381  ACGTTGGCTCAGCTGGTCATTCAACAGCAACACCAGCAATTCTTGGAGAAGCAGAAGCAA 1440
 461  T  L  A  Q  L  V  I  Q  Q  Q  H  Q  Q  F  L  E  K  Q  K  Q  480
```

FIG. 15A

```
1441  TACCAGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTGAAGCAA  1500
 481  Y  Q  Q  Q  I  H  M  N  K  L  L  S  K  S  I  E  Q  L  K  Q    500
1501  CCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGACCAGGCGATGCAGGAA  1560
 501  P  G  S  H  L  E  E  A  E  E  E  L  Q  G  D  Q  A  M  Q  E    520
1561  GACAGAGCGCCCTCTAGTGGCAACAGCACTAGGAGCGACAGCAGTGCTTGTGTGGATGAC  1620
 521  D  R  A  P  S  S  G  N  S  T  R  S  D  S  S  A  C  V  D  D    540
1621  ACACTGGGACAAGTTGGGGCTGTGAAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGAT  1680
 541  T  L  G  Q  V  G  A  V  K  V  K  E  E  P  V  D  S  D  E  D    560
1681  GCTCAGATCCAGGAAATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGCCTTTCCTG  1740
 561  A  Q  I  Q  E  M  E  S  G  E  Q  A  A  F  M  Q  Q  P  F  L    580
1741  GAACCCACGCACACACGTGCGCTCTCTGTGCGCCAAGCTCCGCTGGCTGCGGTTGGCATG  1800
 581  E  P  T  H  T  R  A  L  S  V  R  Q  A  P  L  A  A  V  G  M    600
1801  GATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCACTCTTCCCCTGCTGCCTCTGTT  1860
 601  D  G  L  E  K  H  R  L  V  S  R  T  H  S  S  P  A  A  S  V    620
1861  TTACCTCACCCGGCAATGGACCGCCCCCTCCAGCCTGGCTCTGCAACTGGAATTGCCTAT  1920
 621  L  P  H  P  A  M  D  R  P  L  Q  P  G  S  A  T  G  I  A  Y    640
1921  GACCCCTTGATGCTGAAACACCAGTGCGTTTGTGGCAATTCCACCACCCACCCTGAGCAT  1980
 641  D  P  L  M  L  K  H  Q  C  V  C  G  N  S  T  T  H  P  E  H    660
1981  GCTGGACGAATACAGAGTATCTGGTCACGACTGCAAGAAACTGGGCTGCTAAATAAATGT  2040
 661  A  G  R  I  Q  S  I  W  S  R  L  Q  E  T  G  L  L  N  K  C    680
2041  GAGCGAATTCAAGGTCGAAAAGCCAGCCTGGAGGAAATACAGCTTGTTCATTCTGAACAT  2100
 681  E  R  I  Q  G  R  K  A  S  L  E  E  I  Q  L  V  H  S  E  H    700
2101  CACTCACTGTTGTATGGCACCAACCCCCTGGACGGACAGAAGCTGGACCCCAGGATACTC  2160
 701  H  S  L  L  Y  G  T  N  P  L  D  G  Q  K  L  D  P  R  I  L    720
2161  CTAGGTGATGACTCTCAAAAGTTTTTTTCCTCATTACCTTGTGGTGGACTTGGGGTGGAC  2220
 721  L  G  D  D  S  Q  K  F  F  S  S  L  P  C  G  G  L  G  V  D    740
2221  AGTGACACCATTTGGAATGAGCTACACTCGTCCGGTGCTGCACGCATGGCTGTTGGCTGT  2280
 741  S  D  T  I  W  N  E  L  H  S  S  G  A  A  R  M  A  V  G  C    760
2281  GTCATCGAGCTGGCTTCCAAAGTGGCCTCAGGAGAGCTGAAGAATGGGTTTGCTGTTGTG  2340
 761  V  I  E  L  A  S  K  V  A  S  G  E  L  K  N  G  F  A  V  V    780
2341  AGGCCCCCTGGCCATCACGCTGAAGAATCCACAGCCATGGGGTTCTGCTTTTTTAATTCA  2400
 781  R  P  P  G  H  H  A  E  E  S  T  A  M  G  F  C  F  F  N  S    800
2401  GTTGCAATTACCGCCAAATACTTGAGAGACCAACTAAATATAAGCAAGATATTGATTGTA  2460
 801  V  A  I  T  A  K  Y  L  R  D  Q  L  N  I  S  K  I  L  I  V    820
2461  GATCTGGATGTTCACCATGGAAACGGTACCCAGCAGGCCTTTTATGCTGACCCCAGCATC  2520
 821  D  L  D  V  H  H  G  N  G  T  Q  Q  A  F  Y  A  D  P  S  I    840
2521  CTGTACATTTCACTCCATCGCTATGATGAAGGGAACTTTTTCCCTGGCAGTGGAGCCCCA  2580
 841  L  Y  I  S  L  H  R  Y  D  E  G  N  F  F  P  G  S  G  A  P    860
2581  AATGAGGTTGGAACAGGCCTTGGAGAAGGGTACAATATAAATATTGCCTGGACAGGTGGC  2640
 861  N  E  V  G  T  G  L  G  E  G  Y  N  I  N  I  A  W  T  G  G    880
2641  CTTGATCCTCCCATGGGAGATGTTGAGTACCTTGAAGCATTCAGGACCATCGTGAAGCCT  2700
 881  L  D  P  P  M  G  D  V  E  Y  L  E  A  F  R  T  I  V  K  P    900
2701  GTGGCCAAAGAGTTTGATCCAGACATGGTCTTAGTATCTGCTGGATTTGATGCATTGGAA  2760
 901  V  A  K  E  F  D  P  D  M  V  L  V  S  A  G  F  D  A  L  E    920
2761  GGCCACACCCCTCCTCTAGGAGGGTACAAAGTGACGGCAAAATGTTTTGGTCATTTGACG  2820
 921  G  H  T  P  P  L  G  G  Y  K  V  T  A  K  C  F  G  H  L  T    940
2821  AAGCAATTGATGACATTGGCTGATGGACGTGTGGTGTTGGCTCTAGAAGGAGGACATGAT  2880
 941  K  Q  L  M  T  L  A  D  G  R  V  V  L  A  L  E  G  G  H  D    960
```

FIG. 15B

```
2881  CTCACAGCCATCTGTGATGCATCAGAAGCCTGTGTAAATGCCCTTCTAGGAAATGAGCTG   2940
 961  L  T  A  I  C  D  A  S  E  A  C  V  N  A  L  L  G  N  E  L    980

2941  GAGCCACTTGCAGAAGATATTCTCCACCAAAGCCCGAATATGAATGCTGTTATTTCTTTA   3000
 981  E  P  L  A  E  D  I  L  H  Q  S  P  N  M  N  A  V  I  S  L   1000

3001  CAGAAGATCATTGAAATTCAAAGCAAGTATTGGAAGTCAGTAAGGATGGTGGCTGTGCCA   3060
1001  Q  K  I  I  E  I  Q  S  K  Y  W  K  S  V  R  M  V  A  V  P   1020

3061  AGGGGCTGTGCTCTGGCTGGTGCTCAGTTGCAAGAGGAGACAGAGACCGTTTCTGCCCTG   3120
1021  R  G  C  A  L  A  G  A  Q  L  Q  E  E  T  E  T  V  S  A  L   1040

3121  GCCTCCCTAACAGTGGATGTGGAACAGCCCTTTGCTCAGGAAGACAGCAGAACTGCTGGT   3180
1041  A  S  L  T  V  D  V  E  Q  P  F  A  Q  E  D  S  R  T  A  G   1060

3181  GAGCCTATGGAAGAGGAGCCAGCCTTGTGAAGTGCCAAGTCCCCCTCTGATATTTCCTGT   3240
1061  E  P  M  E  E  E  P  A  L                                     1069

3241  GTGTGACATCATTGTGTATCCCCCCACCCCAGTACCCTCAGACATGTCTTGTCTGCTGCC   3300
3301  TGGGTGGCACAGATTCAATGGAACATAAACACTGGGCACAAAATTCTGAACAGCAGCTTC   3360
3361  ACTTGTTCTTTGGATGGACTTGAAAGGGCATTAAAGATTCCTTAAACGTAACCGCTGTGA   3420
3421  TTCTAGAGTTACAGTAAACCACGATTGGAAGAAACTGCTTCCAGCATGCTTTTAATATGC   3480
3481  TGGGTGACCCACTCCTAGACACCAAGTTTGAACTAGAAACATTCAGTACAGCACTAGATA   3540
3541  TTGTTAATTTCAGAAGCTATGACAGCCAGTGAAATTTTGGGCAAAACCTGAGACATAGTC   3600
3601  ATTCCTGACATTCTGATCAGCTTTTTTTGGGGTAATTTGTTTTTCAAACAGTCTTAACTT   3660
3661  GTTTACAAGATTTGCTTTTAGCTATGAACGGATCGTAATTCCACCCAGAATGTAATGTTT   3720
3721  CTTGTTTGTTTGTTTTGTTTTGTTAGGGTTTTTTTTCTCAACTTTAACACACAGTTCAACT   3780
3781  GTTCCTAGTAAAAGTTCAAGATGGAGGAACTAGCATGAGGCTTTTTTCAGTATCTCGAAG   3840
3841  TCCAAATGCCAAAGGAACCTCACACACTGTTTGTAATGGTGCAATATTTTATATCACTTT   3900
3901  TTTTTAAACATCCCCAACATCTTTGTGTTCTCACACACAGGCAATTTGCAATGTTGCAAT   3960
3961  TGTGTTGGAGAATGAAGTCCCCCCACCTCCCAGCCACACACACATCCTTTGTTCTCATGA   4020
4021  CAGTAGGTCTGAGCAAATGTTCCACCAAGCATTTTCAGTGTCTTTGAAAAGCACGTAACT   4080
4081  TTTCAAAGGTGGTCTTAATTTGCTGCATATCTATCAAGGACTTATTCACTCACCTTTCCT   4140
4141  TTTCTGCCCTCTATCAATTGATTTCTTCTTACCTTTCATCATTCATTCCTTCCTTTAGAA   4200
4201  AAACTGAAGATTACCCATAATCTCCTCTTATTACTTGAGGGCCTTGACTATTTAGTTTAT   4260
4261  TTTGTTTACTTTACAGGTTAACACAGTTGTTTTGTCTGATTGCATTTTATTAACTGTGAA   4320
4321  GCCGTTGAAATGAATATCACTTAAGCAACGTTGCTAAATTTCTATGTGTTTGAAATGTGT   4380
4381  TAATGAAGGCACTGCTTATTTGTAGTCACCTTGAACTGACTTAACCTAGAAGCTGTGCCT   4440
4441  TCTTGTGAAAAAAAAAAAAAAAAAAA  4467
```

FIG. 15C

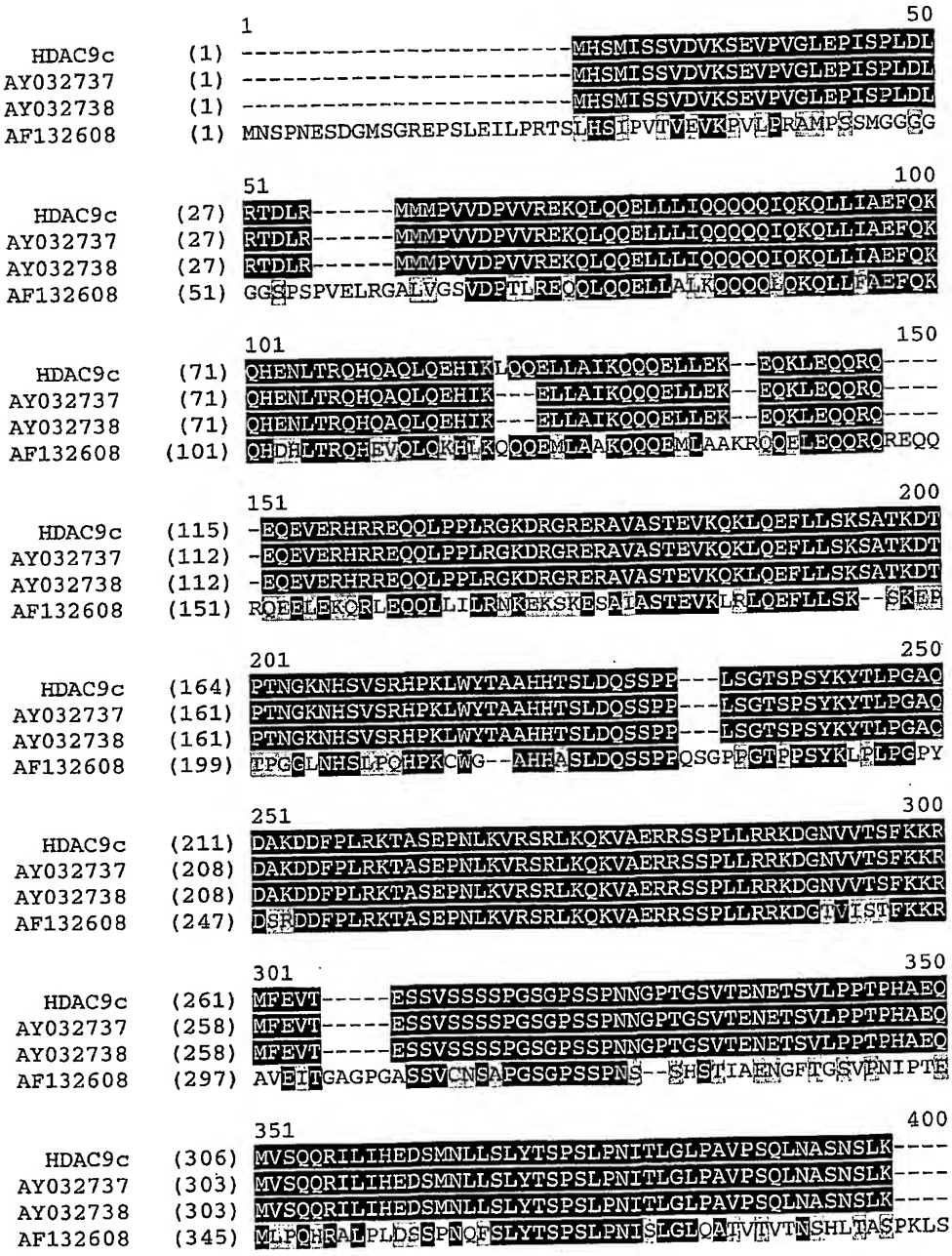



FIG. 15D

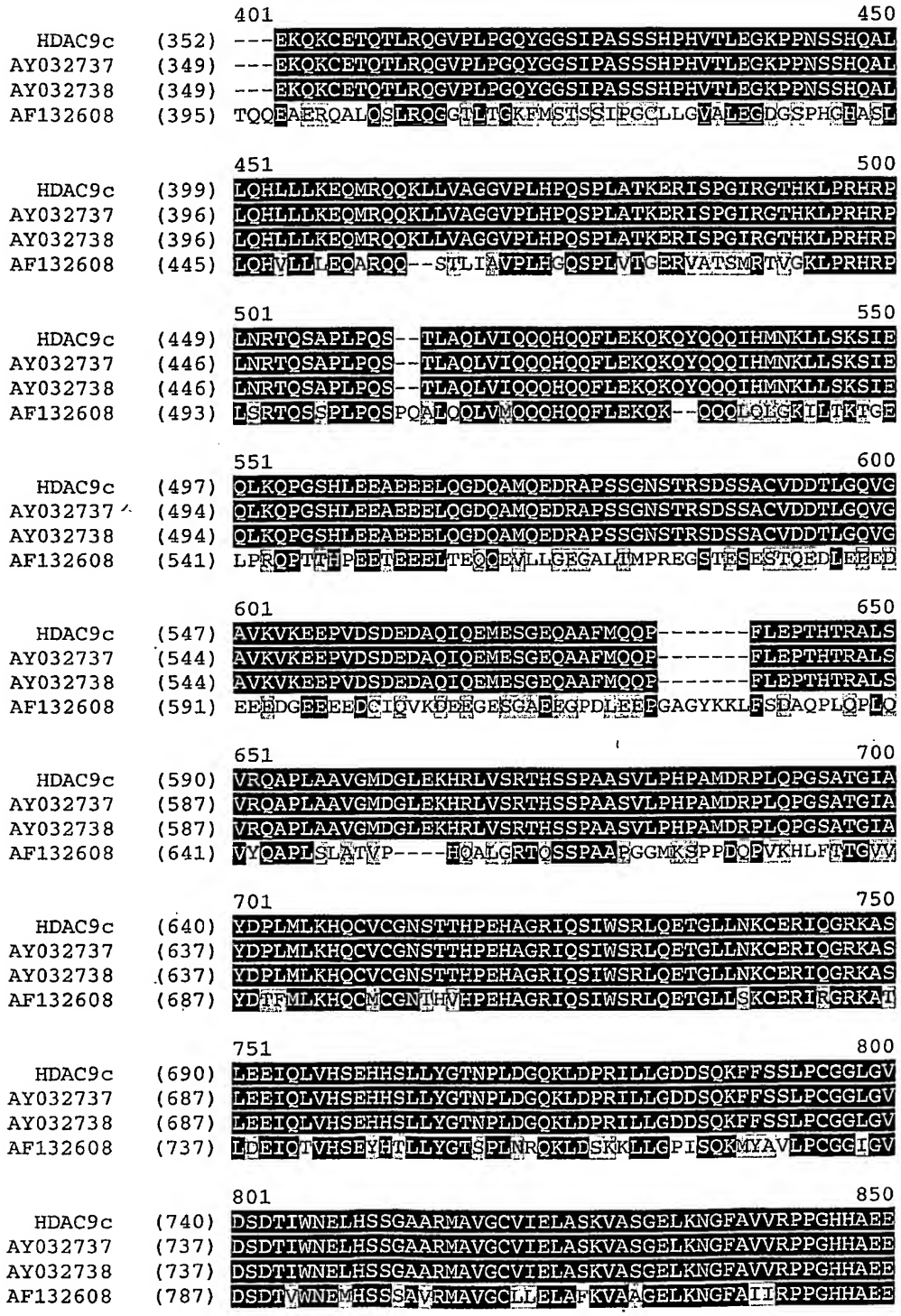



FIG. 15E

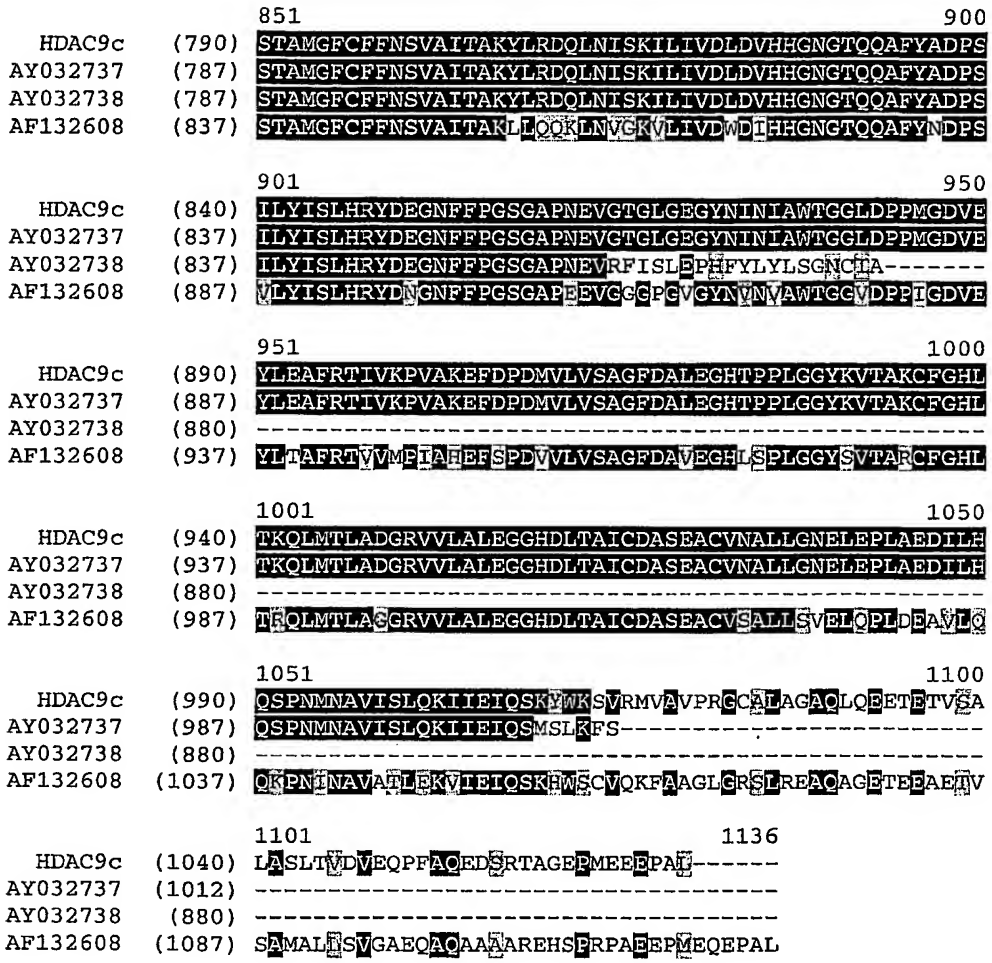



FIG. 15F

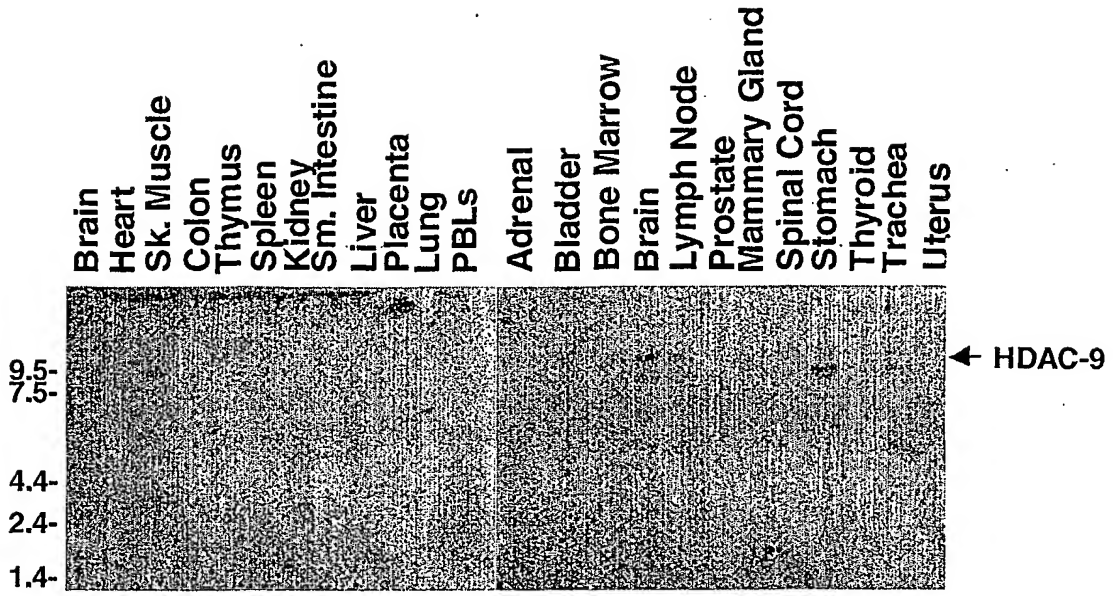



FIG. 16A

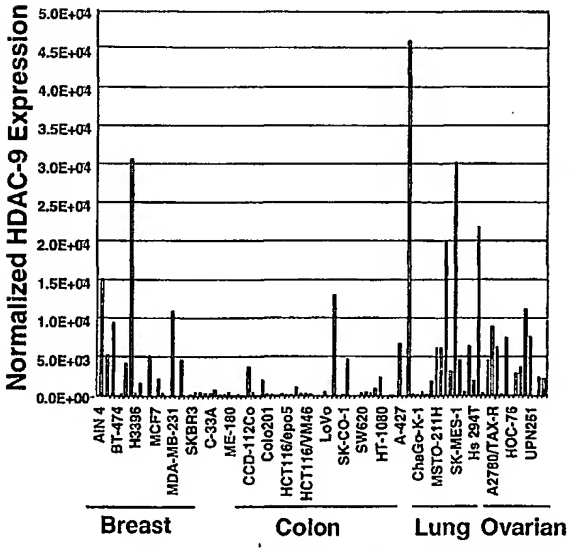



FIG. 16B

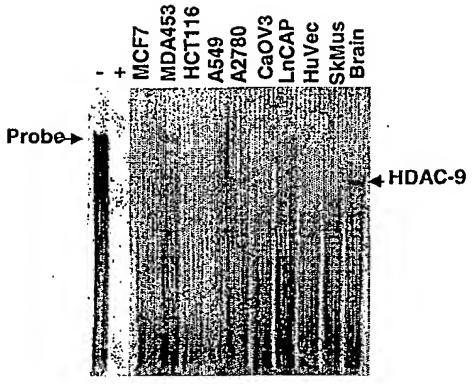



FIG. 16C

```
2901                                        GG AAATGAGCTG GAGCCACTTG
2951  CAGAAGATAT TCTCCACCAA AGCCCGAATA TGAATGCTGT TATTTCTTTA
3001  CAGAAGATCA TTGAAATTCA AAGCAAGTAT TGGAAGTCAG TAAGGATGGT
3051  GGCTGTGCCA AGGGGCTGTG CTCTGGCTGG TGCTCAGTTG CAAGAGGAGA
3101  CAGAGACCGT TTCTGCCCTG GCCTCCCTAA CAGTGGATGT GGAACAGCCC
3151  TTTGCTCAGG AAGACAGCAG AACTGCTGGT GAGCCTATGG AAGAGGAGCC
3201  AGCCTTGTGA
```

FIG. 16D

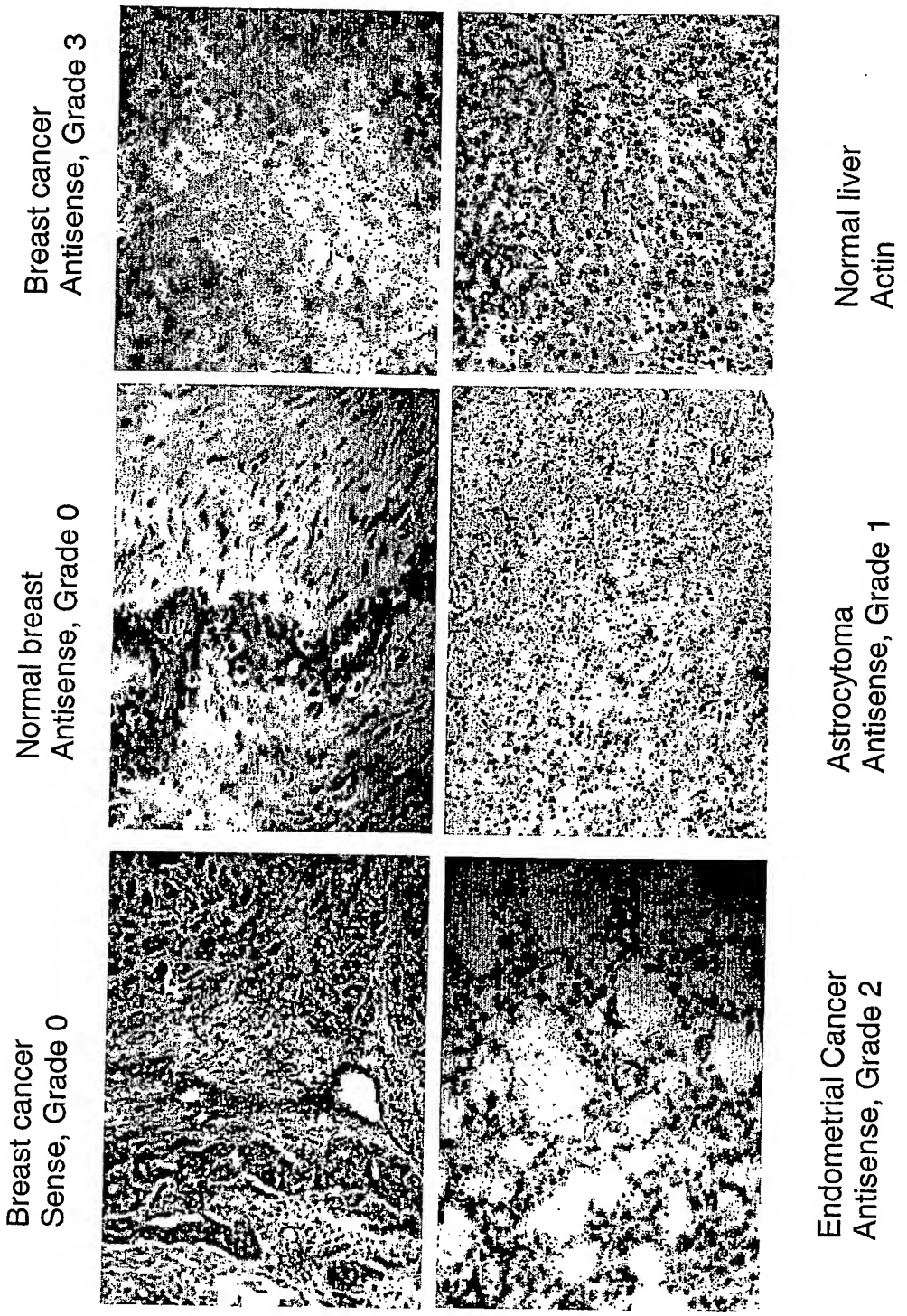
| Tissue Type | Age | Sex | Histology | Surgery | Resected Margin | Stage | HDAC-9/X | b-Actin |
|---|---|---|---|---|---|---|---|---|
| Breast | Unk | F | Infiltrating ductal adenocarcinoma | Mastectomy | Positive | 2 | +4 | 0 |
| Breast | 72 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 3 | +2 | +1 |
| Breast | 81 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 3 | NA | +1 |
| Breast | 43 | F | Infiltrating ductal adenocarcinoma | Mastectomy | | 0 | +2 | +1 |
| Breast | 61 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 2 | +2 | +1 |
| Breast | 77 | F | Infiltrating ductal adenocarcinoma | Mastectomy | | 3 | +2 | +1 |
| Breast | 69 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Positive | 3 | +3 | +1 |
| Breast | 76 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 2 | +2 | +1 |
| Breast | Unk | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 2 | +4 | +1 |
| Breast | 44 | F | Infiltrating ductal adenocarcinoma | Mastectomy | | 3 | +2 | 0 |
| Breast | 61 | F | Infiltrating ductal adenocarcinoma | Mastectomy | Negative | 2 | +2 | +1 |
| Breast | 46 | F | Infiltrating ductal adenocarcinoma | Mastectomy | | 3 | +2 | 0 |
| Breast | 86 | F | Infiltrating ductal adenocarcinoma | Biopsy | | 3 | +2 | +1 |
| Breast | 65 | F | Lobular adenocarcinoma | Mastectomy | | 3 | +2 | +1 |
| Breast | 88 | F | Infiltrating ductal adenocarcinoma | Mastectomy | | 3 | +1 | 0 |
| Breast | 47 | F | Infiltrating ductal adenocarcinoma | Biopsy | | 1 | +1 | +1 |
| Prostate | 77 | M | Adenocarcinoma | TUR | | 1 | 0 | +1 |
| Prostate | 74 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 55 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 68 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 71 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 66 | M | Adenocarcinoma | TUR | | 1 | +2 | +1 |
| Prostate | 69 | M | Adenocarcinoma | TUR | | 1 | +2 | +1 |
| Prostate | 73 | M | Adenocarcinoma | TUR | | 1 | +2 | +1 |
| Prostate | 72 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 77 | M | Adenocarcinoma | TUR | | 1 | +4 | +1 |
| Prostate | 77 | M | Adenocarcinoma | TUR | | 1 | +2 | +1 |
| Prostate | 73 | M | Adenocarcinoma | TUR | | 1 | +2 | +1 |
| Prostate | 84 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 93 | M | Adenocarcinoma | TUR | | 1 | +1 | 0 |
| Prostate | 78 | M | Adenocarcinoma | TUR | | 1 | +1 | +1 |
| Prostate | 78 | M | Matched benign specimen | TUR | | | +1 | 0 |

FIG. 17A

| Tissue Type | Age | Sex | Histology | Surgery | Resected Margin | Stage | HDAC-9/X | b-Actin |
|---|---|---|---|---|---|---|---|---|
| Breast | Unk | F | No pathological diagnosis | Biopsy | | | 0 | +1 |
| Breast | Unk | F | No pathological diagnosis | Biopsy | | | 0 | +1 |
| Breast | 43 | F | No pathological diagnosis | Mastectomy | | | 0 | 0 |
| Breast | 88 | F | No pathological diagnosis | Mastectomy | | | 0 | +1 |
| Breast | 55 | F | No pathological diagnosis | Mastectomy | | | 0 | +1 |
| Breast | 74 | F | No pathological diagnosis | Mastectomy | | | +1 | +1 |
| Breast | 51 | F | No pathological diagnosis | Mastectomy | | | +1 | +1 |
| Prostate | 69 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 69 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 66 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 69 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 76 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 64 | M | No pathological diagnosis | TUR | | | 0 | +1 |
| Prostate | 66 | M | No pathological diagnosis | TUR | | | 0 | +1 |

FIG. 17B

FIG. 17C



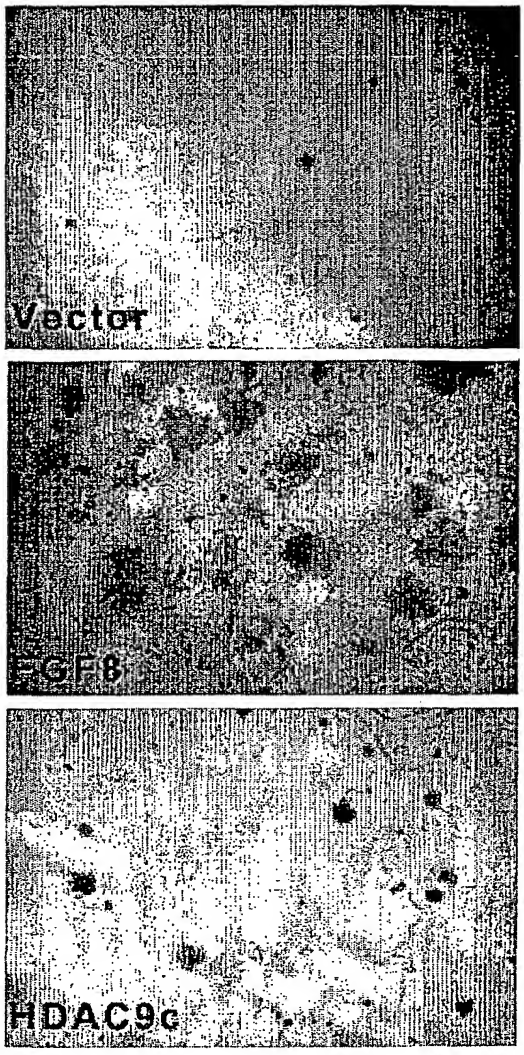



FIG. 18

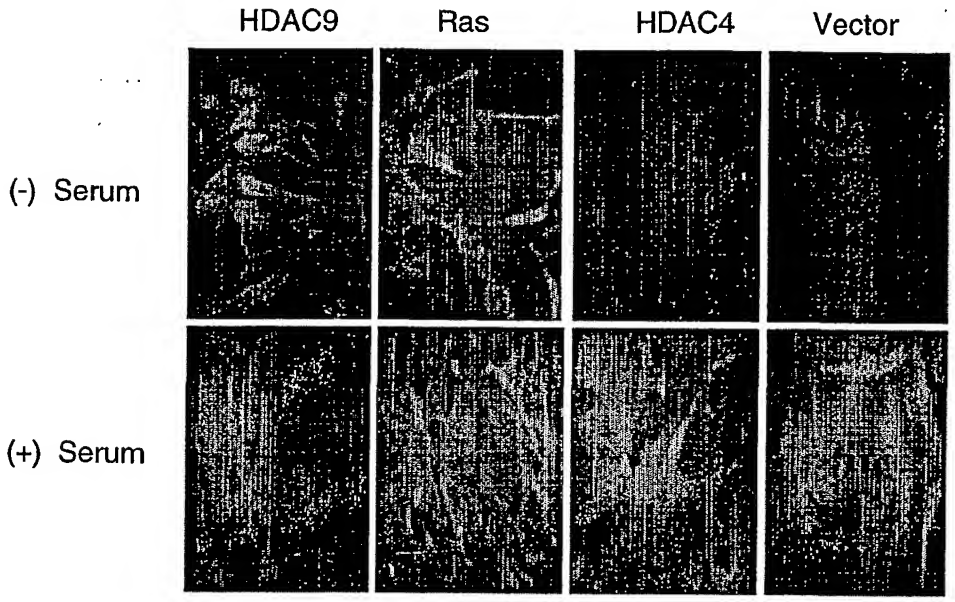



FIG. 19

```
      AlaGluAsnGluThrSerValLeuProProThrProHisAlaGluGlnMetValSerGln
1     GCTGAAAATGAGACTTCGGTTTTGCCCCCTACCCCTCATGCCGAGCAAATGGTTTCACAG

      GlnArgIleLeuIleHisGluAspSerMetAsnLeuLeuSerLeuTyrThrSerProSer
61    CAACGCATTCTAATTCATGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCT

      LeuProAsnIleThrLeuGlyLeuProAlaValProSerGlnLeuAsnAlaSerAsnSer
121   TTGCCCAACATTACCTTGGGGCTTCCCGCAGTGCCATCCCAGCTCAATGCTTCGAATTCA

      LeuLysGluLysGlnLysCysGluThrGlnThrLeuArgGlnGlyValProLeuProGly
181   CTCAAAGAAAAGCAGAAGTGTGAGACGCAGACGCTTAGGCAAGGTGTTCCTCTGCCTGGG

      GlnTyrGlyGlySerIleProAlaSerSerSerHisProHisValThrLeuGluGlyLys
241   CAGTATGGAGGCAGCATCCCGGCATCTTCCAGCCACCCTCATGTTACTTTAGAGGGAAAG

      ProProAsnSerSerHisGlnAlaLeuLeuGlnHisLeuLeuLeuLysGluGlnMetArg
301   CCACCCAACAGCAGCCACCAGGCTCTCCTGCAGCATTTATTATTGAAAGAACAAATGCGA

      GlnGlnLysLeuLeuValAlaGlyGlyValProLeuHisProGlnSerProLeuAlaThr
361   CAGCAAAAGCTTCTTGTAGCTGGTGGAGTTCCCTTACATCCTCAGTCTCCCTTGGCAACA

      LysGluArgIleSerProGlyIleArgGlyThrHisLysLeuProArgHisArgProLeu
421   AAAGAGAGAATTTCACCTGGCATTAGAGGTACCCACAAATTGCCCCGTCACAGACCCCTG

      AsnArgThrGlnSerAlaProLeuProGlnSerThrLeuAlaGlnLeuValIleGlnGln
481   AACCGAACCCAGTCTGCACCTTTGCCTCAGAGCACGTTGGCTCAGCTGGTCATTCAACAG

      GlnHisGlnGlnPheLeuGluLysGlnLysGlnTyrGlnGlnGlnIleHisMetAsnLys
541   CAACACCAGCAATTCTTGGAGAAGCAGAAGCAATACCAGCAGCAGATCCACATGAACAAA

      LeuLeuSerLysSerIleGluGlnLeuLysGlnProGlySerHisLeuGluGluAlaGlu
601   CTGCTTTCGAAATCTATTGAACAACTGAAGCAACCAGGCAGTCACCTTGAGGAAGCAGAG

      GluGluLeuGlnGlyAspGlnAlaMetGlnGluAspArgAlaProSerSerGlyAsnSer
661   GAAGAGCTTCAGGGGGACCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGC

      ThrArgSerAspSerSerAlaCysValAspAspThrLeuGlyGlnValGlyAlaValLys
721   ACTAGGAGCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTGAAG

      ValLysGluGluProValAspSerAspGluAspAlaGlnIleGlnGluMetGluSerGly
781   GTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGAAATGGAATCTGGG

      GluGlnAlaAlaPheMetGlnGlnProPheLeuGluProThrHisThrArgAlaLeuSer
841   GAGCAGGCTGCTTTTATGCAACAGCCTTTCCTGGAACCCACGCACACACGTGCGCTCTCT

      ValArgGlnAlaProLeuAlaAlaValGlyMetAspGlyLeuGluLysHisArgLeuVal
901   GTGCGCCAAGCTCCGCTGGCTGCGGTTGGCATGGATGGATTAGAGAAACACCGTCTCGTC

      SerArgThrHisSerSerProAlaAlaSerValLeuProHisProAlaMetAspArgPro
961   TCCAGGACTCACTCTTCCCCTGCTGCCTCTGTTTTACCTCACCCGGCAATGGACCGCCCC

      LeuGlnProGlySerAlaThrGlyIleAlaTyrAspProLeuMetLeuLysHisGlnCys
1021  CTCCAGCCTGGCTCTGCAACTGGAATTGCCTATGACCCCTTGATGCTGAAACACCAGTGC

      ValCysGlyAsnSerThrThrHisProGluHisAlaGlyArgIleGlnSerIleTrpSer
1081  GTTTGTGGCAATTCCACCACCCACCCTGAGCATGCTGGACGAATACAGAGTATCTGGTCA
```

FIG. 20A

```
                ArgLeuGlnGluThrGlyLeuLeuAsnLysCysGluArgIleGlnGlyArgLysAlaSer
1141            CGACTGCAAGAAACTGGGCTGCTAAATAAATGTGAGCGAATTCAAGGTCGAAAAGCCAGC

                LeuGluGluIleGlnLeuValHisSerGluHisHisSerLeuLeuTyrGlyThrAsnPro
1201            CTGGAGGAAATACAGCTTGTTCATTCTGAACATCACTCACTGTTGTATGGCACCAACCCC

                LeuAspGlyGlnLysLeuAspProArgIleLeuLeuGlyAspAspSerGlnLysPhePhe
1261            CTGGACGGACAGAAGCTGGACCCCAGGATACTCCTAGGTGATGACTCTCAAAAGTTTTTT

                SerSerLeuProCysGlyGlyLeuGlyValAspSerAspThrIleTrpAsnGluLeuHis
1321            TCCTCATTACCTTGTGGTGGACTTGGGGTGGACAGTGACACCATTTGGAATGAGCTACAC

                SerSerGlyAlaAlaArgMetAlaValGlyCysValIleGluLeuAlaSerLysValAla
1381            TCGTCCGGTGCTGCACGCATGGCTGTTGGCTGTGTCATCGAGCTGGCTTCCAAAGTGGCC

                SerGlyGluLeuLysAsnGlyPheAlaValValArgProProGlyHisHisAlaGluGlu
1441            TCAGGAGAGCTGAAGAATGGGTTTGCTGTTGTGAGGCCCCCTGGCCATCACGCTGAAGAA

                SerThrAlaMetGlyPheCysPhePheAsnSerValAlaIleThrAlaLysTyrLeuArg
1501            TCCACAGCCATGGGGTTCTGCTTTTTTAATTCAGTTGCAATTACCGCCAAATACTTGAGA

                AspGlnLeuAsnIleSerLysIleLeuIleValAspLeuAspValHisHisGlyAsnGly
1561            GACCAACTAAATATAAGCAAGATATTGATTGTAGATCTGGATGTTCACCATGGAAACGGT

                ThrGlnGlnAlaPheTyrAlaAspProSerIleLeuTyrIleSerLeuHisArgTyrAsp
1621            ACCCAGCAGGCCTTTTATGCTGACCCCAGCATCCTGTACATTTCACTCCATCGCTATGAT

                GluGlyAsnPhePheProGlySerGlyAlaProAsnGluValGlyThrGlyLeuGlyGlu
1681            GAAGGGAACTTTTTCCCTGGCAGTGGAGCCCCAAATGAGGTTGGAACAGGCCTTGGAGAA

                GlyTyrAsnIleAsnIleAlaTrpThrGlyGlyLeuAspProProMetGlyAspValGlu
1741            GGGTACAATATAAATATTGCCTGGACAGGTGGCCTTGATCCTCCCATGGGAGATGTTGAG

                TyrLeuGluAlaPheArgThrIleValLysProValAlaLysGluPheAspProAspMet
1801            TACCTTGAAGCATTCAGGACCATCGTGAAGCCTGTGGCCAAAGAGTTTGATCCAGACATG

                ValLeuValSerAlaGlyPheAspAlaLeuGluGlyHisThrProProLeuGlyGlyTyr
1861            GTCTTAGTATCTGCTGGATTTGATGCATTGGAAGGCCACACCCCTCCTCTAGGAGGGTAC

                LysValThrAlaLysCysPheGlyHisLeuThrLysGlnLeuMetThrLeuAlaAspGly
1921            AAAGTGACGGCAAAATGTTTTGGTCATTTGACGAAGCAATTGATGACATTGGCTGATGGA

                ArgValValLeuAlaLeuGluGlyGlyHisAspLeuThrAlaIleCysAspAlaSerGlu
1981            CGTGTGGTGTTGGCTCTAGAAGGAGGACATGATCTCACAGCCATCTGTGATGCATCAGAA

                AlaCysValAsnAlaLeuLeuGlyAsnGluLeuGluProLeuAlaGluAspIleLeuHis
2041            GCCTGTGTAAATGCCCTTCTAGGAAATGAGCTGGAGCCACTTGCAGAAGATATTCTCCAC

                GlnSerProAsnMetAsnAlaValIleSerLeuGlnLysIleIleGluIleGlnSerLys
2101            CAAAGCCCGAATATGAATGCTGTTATTTCTTTACAGAAGATCATTGAAATTCAAAGCAAG

                TyrTrpLysSerValArgMetValAlaValProArgGlyCysAlaLeuAlaGlyAlaGln
2161            TATTGGAAGTCAGTAAGGATGGTGGCTGTGCCAAGGGGCTGTGCTCTGGCTGGTGCTCAG

                LeuGlnGluGluThrGluThrValSerAlaLeuAlaSerLeuThrValAspValGluGln
2221            TTGCAAGAGGAGACAGAGACCGTTTCTGCCCTGGCCTCCCTAACAGTGGATGTGGAACAG
```

FIG. 20B

```
        ProPheAlaGlnGluAspSerArgThrAlaGlyGluProMetGluGluGluProAlaLeu
2281    CCCTTTGCTCAGGAAGACAGCAGAACTGCTGGTGAGCCTATGGAAGAGGAGCCAGCCTTG

        ***
2341    TGAAGTGCCAAGTCCCCCTCTGATATTTCCTGTGTGTGACATCATTGTGTATCCCCCCAC

2401    CCCAGTACCCTCAGACATGTCTTGTCTGCTGCCTGGGTGGCACAGATTCAATGGAACATA
2461    AACACTGGGCACAAAATTCTGAACAGCAGCTTCACTTGTTCTTTGGATGGACTTGAAAGG
2521    GCATTAAAGATTCCTTAAACGTAACCGCTGTGATTCTAGAGTTACAGTAAACCACGATTG
2581    GAAGAAACTGCTTCCAGCATGCTTTTAATATGCTGGGTGACCCACTCCTAGACACCAAGT
2641    TTGAACTAGAAACATTCAGTACAGCACTAGATATTGTTAATTTCAGAAGCTATGACAGCC
2701    AGTGAAATTTTGGGCAAAACCTGAGACATAGTCATTCCTGACATTCTGATCAGCTTTTTT
2761    TGGGGTAATTTGTTTTTCAAACAGTCTTAACTTGTTTACAAGATTTGCTTTTAGCTATGA
2821    ACGGATCGTAATTCCACCCAGAATGTAATGTTTCTTGTTTGTTTGTTTTGTTTTGTTAGG
2881    GTTTTTTTCTCAACTTTAACACACAGTTCAACTGTTCCTAGTAAAAGTTCAAGATGGAGG
2941    AACTAGCATGAGGCTTTTTTCAGTATCTCGAAGTCCAAATGCCAAAGGAACCTCACACAC
3001    TGTTTGTAATGGTGCAATATTTTATATCACTTTTTTTTTAAACATCCCCAACATCTTTGTG
3061    TTCTCACACACAGGCAATTTGCAATGTTGCAATTGTGTTGGAGAATGAAGTCCCCCCACC
3121    TCCCAGCCACACACACATCCTTTGTTCTCATGACAGTAGGTCTGAGCAAATGTTCCACCA
3181    AGCATTTTCAGTGTCTTTGAAAAGCACGTAACTTTTCAAAGGTGGTCTTAATTTGCTGCA
3241    TATCTATCAAGGACTTATTCACTCACCTTTCCTTTTCTGCCCTCTATCAATTGATTTCTT
3301    CTTACCTTTCATCATTCATTCCTTCCTTTAGAAAAACTGAAGATTACCCATAATCTCCTC
3361    TTATTACTTGAGGGCCTTGACTATTTAGTTTATTTTGTTTACTTTACAGGTTAACACAGT
3421    TGTTTTGTCTGATTGCATTTTATTAACTGTGAAGCCGTTGAAATGAATATCACTTAAGCA
3481    ACGTTGCTAAATTTCTATGTGTTTGAAATGTGTTAATGAAGGCACTGCTTATTTGTAGTC
3541    ACCTTGAACTGACTTAACCTAGAAGCTGTGCCTTCTTGTGAAAAAAAAAAAAAAAAAAAA
3601    AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

FIG. 20C

```
   1  CCACGCGTCCGTAGGAGAAGGGCACCGGCTGGAGCCACTTGCAGGACTGAGGGTTTTTGC
  61  AACAAAACCCTAGCAGCCTGAAGAACTCTAAGCCAGGTTTAATTGGTTTCTTTTTCTCGT
 121  GGGTAGACTTAATAATTTTCTACGTATTCTGACAAAGAAATAACCCCGAAGCACGTTCCT
 181  ATTTCCCACCTGCTTGTAGTTTCCGGGATAACCTAAACTCCAGAGAGCTATAGCATCCAC
 241  TCTGTCCTTTCTGCTTTGCACACAGATGGGGTGGCTGGACGAGAGCAGCTCTTGGCTCAG

                MetHisSerMetIleSerSerValAspValLysSerGluValProValGlyLeu
 301  CAAAGAATGCACAGTATGATCAGCTCAGTGGATGTGAAGTCAGAAGTTCCTGTGGGCCTG

      GluProIleSerProLeuAspLeuArgThrAspLeuArgMetMetMetProValValAsp
 361  GAGCCCATCTCACCTTTAGACCTAAGGACAGACCTCAGGATGATGATGCCCGTGGTGGAC

      ProValValArgGluLysGlnLeuGlnGlnGluLeuLeuLeuIleGlnGlnGlnGlnGln
 421  CCTGTTGTCCGTGAGAAGCAATTGCAGCAGGAATTACTTCTTATCCAGCAGCAGCAACAA

      IleGlnLysGlnLeuLeuIleAlaGluPheGlnLysGlnHisGluAsnLeuThrArgGln
 481  ATCCAGAAGCAGCTTCTGATAGCAGAGTTTCAGAAACAGCATGAGAACTTGACACGGCAG

      HisGlnAlaGlnLeuGlnGluHisIleLysLeuGlnGlnGluLeuLeuAlaIleLysGln
 541  CACCAGGCTCAGCTTCAGGAGCATATCAAGTTGCAACAGGAACTTCTAGCCATAAAACAG

      GlnGlnGluLeuLeuGluLysGluGlnLysLeuGluGlnGlnArgGlnGluGlnGluVal
 601  CAACAAGAACTCCTAGAAAAGGAGCAGAAACTGGAGCAGCAGAGGCAAGAACAGGAAGTA

      GluArgHisArgArgGluGlnGlnLeuProProLeuArgGlyLysAspArgGlyArgGlu
 661  GAGAGGCATCGCAGAGAACAGCAGCTTCCTCCTCTCAGAGGCAAAGATAGAGGACGAGAA

      ArgAlaValAlaSerThrGluValLysGlnLysLeuGlnGluPheLeuLeuSerLysSer
 721  AGGGCAGTGGCAAGTACAGAAGTAAAGCAGAAGCTTCAAGAGTTCCTACTGAGTAAATCA

      AlaThrLysAspThrProThrAsnGlyLysAsnHisSerValSerArgHisProLysLeu
 781  GCAACGAAAGACACTCCAACTAATGGAAAAAATCATTCCGTGAGCCGCCATCCCAAGCTC

      TrpTyrThrAlaAlaHisHisThrSerLeuAspGlnSerSerProProLeuSerGlyThr
 841  TGGTACACGGCTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA

      SerProSerTyrLysTyrThrLeuProGlyAlaGlnAspAlaLysAspAspPheProLeu
 901  TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGATTTCCCCCTT

      ArgLysThrAlaSerGluProAsnLeuLysValArgSerArgLeuLysGlnLysValAla
 961  CGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCAGGTTAAAACAGAAAGTGGCA

      GluArgArgSerSerProLeuLeuArgArgLysAspGlyAsnValValThrSerPheLys
1021  GAGAGGAGAAGCAGCCCCTTACTCAGGCGGAAGGATGGAAATGTTGTCACTTCATTCAAG

      LysArgMetPheGluValThrGluSerSerValSerSerSerSerProGlySerGlyPro
1081  AAGCGAATGTTTGAGGTGACAGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCC

      SerSerProAsnAsnGlyProThrGlySerValThrGluAsnGluThrSerValLeuPro
1141  AGTTCACCAAACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC

      ProThrProHisAlaGluGlnMetValSerGlnGlnArgIleLeuIleHisGluAspSer
1201  CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCATGAAGATTCC

      MetAsnLeuLeuSerLeuTyrThrSerProSerLeuProAsnIleThrLeuGlyLeuPro
1261  ATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCAACATTACCTTGGGGCTTCCC
```

FIG. 21A

```
      AlaValProSerGlnLeuAsnAlaSerAsnSerLeuLysGluLysGlnLysCysGluThr
1321  GCAGTGCCATCCCAGCTCAATGCTTCGAATTCACTCAAAGAAAAGCAGAAGTGTGAGACG

      GlnThrLeuArgGlnGlyValProLeuProGlyGlnTyrGlyGlySerIleProAlaSer
1381  CAGACGCTTAGGCAAGGTGTTCCTCTGCCTGGGCAGTATGGAGGCAGCATCCCGGCATCT

      SerSerHisProHisValThrLeuGluGlyLysProProAsnSerSerHisGlnAlaLeu
1441  TCCAGCCACCCTCATGTTACTTTAGAGGGAAAGCCACCCAACAGCAGCCACCAGGCTCTC

      LeuGlnHisLeuLeuLeuLysGluGlnMetArgGlnGlnLysLeuLeuValAlaGlyGly
1501  CTGCAGCATTTATTATTGAAAGAACAAATGCGACAGCAAAAGCTTCTTGTAGCTGGTGGA

      ValProLeuHisProGlnSerProLeuAlaThrLysGluArgIleSerProGlyIleArg
1561  GTTCCCTTACATCCTCAGTCTCCCTTGGCAACAAAAGAGAGAATTTCACCTGGCATTAGA

      GlyThrHisLysLeuProArgHisArgProLeuAsnArgThrGlnSerAlaProLeuPro
1621  GGTACCCACAAATTGCCCCGTCACAGACCCCTGAACCGAACCCAGTCTGCACCTTTGCCT

      GlnSerThrLeuAlaGlnLeuValIleGlnGlnGlnHisGlnGlnPheLeuGluLysGln
1681  CAGAGCACGTTGGCTCAGCTGGTCATTCAACAGCAACACCAGCAATTCTTGGAGAAGCAG

      LysGlnTyrGlnGlnGlnIleHisMetAsnLysGluLeuProMetThrPro***
1741  AAGCAATACCAGCAGCAGATCCACATGAACAAAGAATTGCCTATGACCCCTTGATGCTGA

1801  AACACCAGTGCGTTTGTGGCAATTCCACCACCCACCCTGAGCATGCTGGACGAATACAGA
1861  GTATCTGGTCACGACTGCAAGAAACTGGGCTGCTAAATAAATGTGAGCGAATTCAAGGTC
1921  GAAAAGCCAGCCTGGAGGAAATACAGCTTGTTCATTCTGAACATCACTCACTGTTGTATG
1981  GCACCAACCCCCTGGACGGACAGAAGCTGGACCCCAGGATACTCCTAGGTGATGACTCTC
2041  AAAAGTTTTTTTCCTCATTACCTTGTGGTGGACTTGGGGTGGACAGTGACACCATTTGGA
2101  ATGAGCTACACTCGTCCGGTGCTGCACGCATGGCTGTTGGCTGTGTCATCGAGCTGGCTT
2161  CCAAAGTGGCCTCAGGAGAGCTGAAGGTGAGGTCCGGGTTGCATTAAGTGTGGGAAATCC
2221  AGAGAAGAAACTGAAACAGAGATGTTGTTATGTGGGAATTGCGGGGAGTGTGGCGTGGTA
2281  ATAAAAGGAAGGGCAGAAGGAAGAGGGTAGAGATGGCCACTAAGGTGTGATAATAACTCA
2341  TCTGTAGGCAGGGAGCAGCTCATCCTGCTCTCAGGGCCTTCTTCTGCCTGAGAACACTCT
2401  GCAGTCAGGGCCCACCGGTGTGCATGTAAGAGCACAGAGATAATAAGCAAAGCTATGGTT
2461  CAGGTTAAAAATACCTTTAGTATATACATGTCTGTCATGCCATCCTGAGATTCTCTTTTG
2521  AGGCAATTTTAAAAATATGATTACTGAGAAGTGTGTATAAGCTCAGAATACCACCCAGAG
2581  AGAGGGAGGCAGAGAAAGGTAAATACCAGACGGGAAGGATTGGGAGGAGGAAGGAAATTG
2641  TTGATTAGAAGGGTAATGATCCAGAGTGTGTTTTTCCATGAAAGAACTTAAAAAATGAGC
2701  TATGCTTTATTGTTCTTTTCTTTTTATGGTCTCTTCTTTTCTACATCGTATGAAAAGAAC
2761  AATGTCCAAACCCCAGCGTTTCCCAGTCTAAACAATTTATAAAAGCTAGAGACCTGACAG
2821  ACGTTGACATTTTATTTGGTATTTTAACAGTGCTATTTAAAGGTACGCCATGTGCGTCTT
2881  GAATGCAGTTACCCCAATAAACTTTGTTGGTGCTAACACGGCCTTTTAATGCACTAGTTC
2941  ACACACTTCATGACGCAATCTGGGTCGTGATTGATTCGGTATTTTTAGCAATTGCGGGGC
3001  TTAGGGAAATATATTATGACCAATAACATATGCACTGTGAGTTTTGTGAAACCAAGATAA
3061  AATAATTAGGATTACTTTTCTTTATGTCTAGTGAATTTTTATTCAATTACATGGGACTCT
3121  TCCAGTTGTGATTAAAAATGTGGAGTAGGAATGTGCACTTCACAATGCAACGTTTGTCCA
3181  AGAAGTCTTTACTCTTAACTCTTTAAAGAGTCAGAGCCTACGGAAATATAATTTTGATAG
3241  GGTGAGCTCTATTTAAAAAGTAGATGTGCCTGTATATATTTGACATAAGTAGTATTAGGA
3301  CATTGCTCATCTCAGGGGATATATGGGGTCATTAATGTGGTGCTTACTCTTCAGTCTTTA
3361  CCTTTGAAAATGAGCAAAAAAAAAAAAAAAA
```

FIG. 21B

```
   1  GGGGAAGAGAGGCACAGACACAGATAGGAGAAGGGCACCGGCTGGAGCCACTTGCAGGAC
  61  TGAGGGTTTTTGCAACAAAACCCTAGCAGCCTGAAGAACTCTAAGCCAGATGGGGTGGCT

                                          MetHisSerMetIleSerSerValAspVal
 121  GGACGAGAGCAGCTCTTGGCTCAGCAAAGAATGCACAGTATGATCAGCTCAGTGGATGTG

      LysSerGluValProValGlyLeuGluProIleSerProLeuAspLeuArgThrAspLeu
 181  AAGTCAGAAGTTCCTGTGGGCCTGGAGCCCATCTCACCTTTAGACCTAAGGACAGACCTC

      ArgMetMetMetProValValAspProValValArgGluLysGlnLeuGlnGlnGluLeu
 241  AGGATGATGATGCCCGTGGTGGACCCTGTTGTCCGTGAGAAGCAATTGCAGCAGGAATTA

      LeuLeuIleGlnGlnGlnGlnGlnIleGlnLysGlnLeuLeuIleAlaGluPheGlnLys
 301  CTTCTTATCCAGCAGCAGCAACAAATCCAGAAGCAGCTTCTGATAGCAGAGTTTCAGAAA

      GlnHisGluAsnLeuThrArgGlnHisGlnAlaGlnLeuGlnGluHisIleLysGluLeu
 361  CAGCATGAGAACTTGACACGGCAGCACCAGGCTCAGCTTCAGGAGCATATCAAGGAACTT

      LeuAlaIleLysGlnGlnGlnGluLeuLeuGluLysGluGlnLysLeuGluGlnGlnArg
 421  CTAGCCATAAAACAGCAACAAGAACTCCTAGAAAAGGAGCAGAAACTGGAGCAGCAGAGG

      GlnGluGlnGluValGluArgHisArgArgGluGlnGlnLeuProProLeuArgGlyLys
 481  CAAGAACAGGAAGTAGAGAGGCATCGCAGAGAACAGCAGCTTCCTCCTCTCAGAGGCAAA

      AspArgGlyArgGluArgAlaValAlaSerThrGluValLysGlnLysLeuGlnGluPhe
 541  GATAGAGGACGAGAAAGGGCAGTGGCAAGTACAGAAGTAAAGCAGAAGCTTCAAGAGTTC

      LeuLeuSerLysSerAlaThrLysAspThrProThrAsnGlyLysAsnHisSerValSer
 601  CTACTGAGTAAATCAGCAACGAAAGACACTCCAACTAATGGAAAAAATCATTCCGTGAGC

      ArgHisProLysLeuTrpTyrThrAlaAlaHisHisThrSerLeuAspGlnSerSerPro
 661  CGCCATCCCAAGCTCTGGTACACGGCTGCCCACCACACATCATTGGATCAAAGCTCTCCA

      ProLeuSerGlyThrSerProSerTyrLysTyrThrLeuProGlyAlaGlnAspAlaLys
 721  CCCCTTAGTGGAACATCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAG

      AspAspPheProLeuArgLysThrAlaSerGluProAsnLeuLysValArgSerArgLeu
 781  GATGATTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCAGGTTA

      LysGlnLysValAlaGluArgArgSerSerProLeuLeuArgArgLysAspGlyAsnVal
 841  AAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGGAAGGATGGAAATGTT

      ValThrSerPheLysLysArgMetPheGluValThrGluSerSerValSerSerSerSer
 901  GTCACTTCATTCAAGAAGCGAATGTTTGAGGTGACAGAATCCTCAGTCAGTAGCAGTTCT

      ProGlySerGlyProSerSerProAsnAsnGlyProThrGlySerValThrGluAsnGlu
 961  CCAGGCTCTGGTCCCAGTTCACCAAACAATGGGCCAACTGGAAGTGTTACTGAAAATGAG

      ThrSerValLeuProProThrProHisAlaGluGlnMetValSerGlnGlnArgIleLeu
1021  ACTTCGGTTTTGCCCCCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTA

      IleHisGluAspSerMetAsnLeuLeuSerLeuTyrThrSerProSerLeuProAsnIle
1081  ATTCATGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCAACATT

      ThrLeuGlyLeuProAlaValProSerGlnLeuAsnAlaSerAsnSerLeuLysGluLys
1141  ACCTTGGGGCTTCCCGCAGTGCCATCCCAGCTCAATGCTTCGAATTCACTCAAAGAAAAG
```

FIG. 22A

```
      GlnLysCysGluThrGlnThrLeuArgGlnGlyValProLeuProGlyGlnTyrGlyGly
1201  CAGAAGTGTGAGACGCAGACGCTTAGGCAAGGTGTTCCTCTGCCTGGGCAGTATGGAGGC

      SerIleProAlaSerSerSerHisProHisValThrLeuGluGlyLysProProAsnSer
1261  AGCATCCCGGCATCTTCCAGCCACCCTCATGTTACTTTAGAGGGAAAGCCACCCAACAGC

      SerHisGlnAlaLeuLeuGlnHisLeuLeuLeuLysGluGlnMetArgGlnGlnLysLeu
1321  AGCCACCAGGCTCTCCTGCAGCATTTATTATTGAAAGAACAAATGCGACAGCAAAAGCTT

      LeuValAlaGlyGlyValProLeuHisProGlnSerProLeuAlaThrLysGluArgIle
1381  CTTGTAGCTGGTGGAGTTCCCTTACATCCTCAGTCTCCCTTGGCAACAAAAGAGAGAATT

      SerProGlyIleArgGlyThrHisLysLeuProArgHisArgProLeuAsnArgThrGln
1441  TCACCTGGCATTAGAGGTACCCACAAATTGCCCCGTCACAGACCCCTGAACCGAACCCAG

      SerAlaProLeuProGlnSerThrLeuAlaGlnLeuValIleGlnGlnGlnHisGlnGln
1501  TCTGCACCTTTGCCTCAGAGCACGTTGGCTCAGCTGGTCATTCAACAGCAACACCAGCAA

      PheLeuGluLysGlnLysGlnTyrGlnGlnGlnIleHisMetAsnLysLeuLeuSerLys
1561  TTCTTGGAGAAGCAGAAGCAATACCAGCAGCAGATCCACATGAACAAACTGCTTTCGAAA

      SerIleGluGlnLeuLysGlnProGlySerHisLeuGluGluAlaGluGluGluLeuGln
1621  TCTATTGAACAACTGAAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAG

      GlyAspGlnAlaMetGlnGluAspArgAlaProSerSerGlyAsnSerThrArgSerAsp
1681  GGGGACCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGAGCGAC

      SerSerAlaCysValAspAspThrLeuGlyGlnValGlyAlaValLysValLysGluGlu
1741  AGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTGAAGGTCAAGGAGGAA

      ProValAspSerAspGluAspAlaGlnIleGlnGluMetGluSerGlyGluGlnAlaAla
1801  CCAGTGGACAGTGATGAAGATGCTCAGATCCAGGAAATGGAATCTGGGGAGCAGGCTGCT

      PheMetGlnGlnProPheLeuGluProThrHisThrArgAlaLeuSerValArgGlnAla
1861  TTTATGCAACAGCCTTTCCTGGAACCCACGCACACACGTGCGCTCTCTGTGCGCCAAGCT

      ProLeuAlaAlaValGlyMetAspGlyLeuGluLysHisArgLeuValSerArgThrHis
1921  CCGCTGGCTGCGGTTGGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCAC

      SerSerProAlaAlaSerValLeuProHisProAlaMetAspArgProLeuGlnProGly
1981  TCTTCCCCTGCTGCCTCTGTTTTACCTCACCCAGCAATGGACCGCCCCCTCCAGCCTGGC

      SerAlaThrGlyIleAlaTyrAspProLeuMetLeuLysHisGlnCysValCysGlyAsn
2041  TCTGCAACTGGAATTGCCTATGACCCCTTGATGCTGAAACACCAGTGCGTTTGTGGCAAT

      SerThrThrHisProGluHisAlaGlyArgIleGlnSerIleTrpSerArgLeuGlnGlu
2101  TCCACCACCCACCCTGAGCATGCTGGACGAATACAGAGTATCTGGTCACGACTGCAAGAA

      ThrGlyLeuLeuAsnLysCysGluArgIleGlnGlyArgLysAlaSerLeuGluGluIle
2161  ACTGGGCTGCTAAATAAATGTGAGCGAATTCAAGGTCGAAAAGCCAGCCTGGAGGAAATA

      GlnLeuValHisSerGluHisHisSerLeuLeuTyrGlyThrAsnProLeuAspGlyGln
2221  CAGCTTGTTCATTCTGAACATCACTCACTGTTGTATGGCACCAACCCCCTGGACGGACAG

      LysLeuAspProArgIleLeuLeuGlyAspAspSerGlnLysPhePheSerSerLeuPro
2281  AAGCTGGACCCCAGGATACTCCTAGGTGATGACTCTCAAAAGTTTTTTTCCTCATTACCT
```

FIG. 22B

```
      CysGlyGlyLeuGlyValAspSerAspThrIleTrpAsnGluLeuHisSerSerGlyAla
2341  TGTGGTGGACTTGGGGTGGACAGTGACACCATTTGGAATGAGCTACACTCGTCCGGTGCT

      AlaArgMetAlaValGlyCysValIleGluLeuAlaSerLysValAlaSerGlyGluLeu
2401  GCACGCATGGCTGTTGGCTGTGTCATCGAGCTGGCTTCCAAAGTGGCCTCAGGAGAGCTG

      LysAsnGlyPheAlaValValArgProProGlyHisHisAlaGluGluSerThrAlaMet
2461  AAGAATGGGTTTGCTGTTGTGAGGCCCCCTGGCCATCACGCTGAAGAATCCACAGCCATG

      GlyPheCysPhePheAsnSerValAlaIleThrAlaLysTyrLeuArgAspGlnLeuAsn
2521  GGGTTCTGCTTTTTTAATTCAGTTGCAATTACCGCCAAATACTTGAGAGACCAACTAAAT

      IleSerLysIleLeuIleValAspLeuAspValHisHisGlyAsnGlyThrGlnGlnAla
2581  ATAAGCAAGATATTGATTGTAGATCTGGATGTTCACCATGGAAACGGTACCCAGCAGGCC

      PheTyrAlaAspProSerIleLeuTyrIleSerLeuHisArgTyrAspGluGlyAsnPhe
2641  TTTTATGCTGACCCCAGCATCCTGTACATTTCACTCCATCGCTATGATGAAGGGAACTTT

      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      PheProGlySerGlyAlaProAsnGluValGlyThrGlyLeuGlyGluGlyTyrAsnIle
2701  TTCCCTGGCAGTGGAGCCCCAAATGAGGTTGGAACAGGCCTTGGAGAAGGGTACAATATA

      AsnIleAlaTrpThrGlyGlyLeuAspProProMetGlyAspValGluTyrLeuGluAla
2761  AATATTGCCTGGACAGGTGGCCTTGATCCTCCCATGGGAGATGTTGAGTACCTTGAAGCA

      PheArgThrIleValLysProValAlaLysGluPheAspProAspMetValLeuValSer
2821  TTCAGGACCATCGTGAAGCCTGTGGCCAAAGAGTTTGATCCAGACATGGTCTTAGTATCT

      AlaGlyPheAspAlaLeuGluGlyHisThrProProLeuGlyGlyTyrLysValThrAla
2881  GCTGGATTTGATGCATTGGAAGGCCACACCCCTCCTCTAGGAGGGTACAAAGTGACGGCA

      LysCysPheGlyHisLeuThrLysGlnLeuMetThrLeuAlaAspGlyArgValValLeu
2941  AAATGTTTTGGTCATTTGACGAAGCAATTGATGACATTGGCTGATGGACGTGTGGTGTTG

      AlaLeuGluGlyGlyHisAspLeuThrAlaIleCysAspAlaSerGluAlaCysValAsn
3001  GCTCTAGAAGGAGGACATGATCTCACAGCCATCTGTGATGCATCAGAAGCCTGTGTAAAT

      AlaLeuLeuGlyAsnGluLeuGluProLeuAlaGluAspIleLeuHisGlnSerProAsn
3061  GCCCTTCTAGGAAATGAGCTGGAGCCACTTGCAGAAGATATTCTCCACCAAAGCCCGAAT

      MetAsnAlaValIleSerLeuGlnLysIleIleGluIleGlnSerMetSerLeuLysPhe
3121  ATGAATGCTGTTATTTCTTTACAGAAGATCATTGAAATTCAAAGTATGTCTTTAAAGTTC

      Ser***
3181  TCTTAA
```

FIG. 22C

```
   1  GGGGAAGAGAGGCACAGACACAGATAGGAGAAGGGCACCGGCTGGAGCCACTTGCAGGAC
  61  TGAGGGTTTTTGCAACAAAACCCTAGCAGCCTGAAGAACTCTAAGCCAGATGGGGTGGCT

                                        MetHisSerMetIleSerSerValAspVal
 121  GGACGAGAGCAGCTCTTGGCTCAGCAAAGAATGCACAGTATGATCAGCTCAGTGGATGTG

      LysSerGluValProValGlyLeuGluProIleSerProLeuAspLeuArgThrAspLeu
 181  AAGTCAGAAGTTCCTGTGGGCCTGGAGCCCATCTCACCTTTAGACCTAAGGACAGACCTC

      ArgMetMetMetProValValAspProValValArgGluLysGlnLeuGlnGlnGluLeu
 241  AGGATGATGATGCCCGTGGTGGACCCTGTTGTCCGTGAGAAGCAATTGCAGCAGGAATTA

      LeuLeuIleGlnGlnGlnGlnGlnIleGlnLysGlnLeuLeuIleAlaGluPheGlnLys
 301  CTTCTTATCCAGCAGCAGCAACAAATCCAGAAGCAGCTTCTGATAGCAGAGTTTCAGAAA

      GlnHisGluAsnLeuThrArgGlnHisGlnAlaGlnLeuGlnGluHisIleLysGluLeu
 361  CAGCATGAGAACTTGACACGGCAGCACCAGGCTCAGCTTCAGGAGCATATCAAGGAACTT

      LeuAlaIleLysGlnGlnGlnGluLeuLeuGluLysGluGlnLysLeuGluGlnGlnArg
 421  CTAGCCATAAAACAGCAACAAGAACTCCTAGAAAAGGAGCAGAAACTGGAGCAGCAGAGG

      GlnGluGlnGluValGluArgHisArgArgGluGlnGlnLeuProProLeuArgGlyLys
 481  CAAGAACAGGAAGTAGAGAGGCATCGCAGAGAACAGCAGCTTCCTCCTCTCAGAGGCAAA

      AspArgGlyArgGluArgAlaValAlaSerThrGluValLysGlnLysLeuGlnGluPhe
 541  GATAGAGGACGAGAAAGGGCAGTGGCAAGTACAGAAGTAAAGCAGAAGCTTCAAGAGTTC

      LeuLeuSerLysSerAlaThrLysAspThrProThrAsnGlyLysAsnHisSerValSer
 601  CTACTGAGTAAATCAGCAACGAAAGACACTCCAACTAATGGAAAAAATCATTCCGTGAGC

      ArgHisProLysLeuTrpTyrThrAlaAlaHisHisThrSerLeuAspGlnSerSerPro
 661  CGCCATCCCAAGCTCTGGTACACGGCTGCCCACCACACATCATTGGATCAAAGCTCTCCA

      ProLeuSerGlyThrSerProSerTyrLysTyrThrLeuProGlyAlaGlnAspAlaLys
 721  CCCCTTAGTGGAACATCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAG

      AspAspPheProLeuArgLysThrAlaSerGluProAsnLeuLysValArgSerArgLeu
 781  GATGATTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCAGGTTA

      LysGlnLysValAlaGluArgArgSerSerProLeuLeuArgArgLysAspGlyAsnVal
 841  AAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGGAAGGATGGAAATGTT

      ValThrSerPheLysLysArgMetPheGluValThrGluSerSerValSerSerSerSer
 901  GTCACTTCATTCAAGAAGCGAATGTTTGAGGTGACAGAATCCTCAGTCAGTAGCAGTTCT

      ProGlySerGlyProSerSerProAsnAsnGlyProThrGlySerValThrGluAsnGlu
 961  CCAGGCTCTGGTCCCAGTTCACCAAACAATGGGCCAACTGGAAGTGTTACTGAAAATGAG

      ThrSerValLeuProProThrProHisAlaGluGlnMetValSerGlnGlnArgIleLeu
1021  ACTTCGGTTTTGCCCCCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTA

      IleHisGluAspSerMetAsnLeuLeuSerLeuTyrThrSerProSerLeuProAsnIle
1081  ATTCATGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCAACATT

      ThrLeuGlyLeuProAlaValProSerGlnLeuAsnAlaSerAsnSerLeuLysGluLys
1141  ACCTTGGGGCTTCCCGCAGTGCCATCCCAGCTCAATGCTTCGAATTCACTCAAAGAAAAG
```

FIG. 22D

```
      GlnLysCysGluThrGlnThrLeuArgGlnGlyValProLeuProGlyGlnTyrGlyGly
1201  CAGAAGTGTGAGACGCAGACGCTTAGGCAAGGTGTTCCTCTGCCTGGGCAGTATGGAGGC

      SerIleProAlaSerSerSerHisProHisValThrLeuGluGlyLysProProAsnSer
1261  AGCATCCCGGCATCTTCCAGCCACCCTCATGTTACTTTAGAGGGAAAGCCACCCAACAGC

      SerHisGlnAlaLeuLeuGlnHisLeuLeuLeuLysGluGlnMetArgGlnGlnLysLeu
1321  AGCCACCAGGCTCTCCTGCAGCATTTATTATTGAAAGAACAAATGCGACAGCAAAAGCTT

      LeuValAlaGlyGlyValProLeuHisProGlnSerProLeuAlaThrLysGluArgIle
1381  CTTGTAGCTGGTGGAGTTCCCTTACATCCTCAGTCTCCCTTGGCAACAAAAGAGAGAATT

      SerProGlyIleArgGlyThrHisLysLeuProArgHisArgProLeuAsnArgThrGln
1441  TCACCTGGCATTAGAGGTACCCACAAATTGCCCCGTCACAGACCCCTGAACCGAACCCAG

      SerAlaProLeuProGlnSerThrLeuAlaGlnLeuValIleGlnGlnGlnHisGlnGln
1501  TCTGCACCTTTGCCTCAGAGCACGTTGGCTCAGCTGGTCATTCAACAGCAACACCAGCAA

      PheLeuGluLysGlnLysGlnTyrGlnGlnGlnIleHisMetAsnLysLeuLeuSerLys
1561  TTCTTGGAGAAGCAGAAGCAATACCAGCAGCAGATCCACATGAACAAACTGCTTTCGAAA

      SerIleGluGlnLeuLysGlnProGlySerHisLeuGluGluAlaGluGluGluLeuGln
1621  TCTATTGAACAACTGAAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAG

      GlyAspGlnAlaMetGlnGluAspArgAlaProSerSerGlyAsnSerThrArgSerAsp
1681  GGGGACCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGAGCGAC

      SerSerAlaCysValAspAspThrLeuGlyGlnValGlyAlaValLysValLysGluGlu
1741  AGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTGAAGGTCAAGGAGGAA

      ProValAspSerAspGluAspAlaGlnIleGlnGluMetGluSerGlyGluGlnAlaAla
1801  CCAGTGGACAGTGATGAAGATGCTCAGATCCAGGAAATGGAATCTGGGGAGCAGGCTGCT

      PheMetGlnGlnProPheLeuGluProThrHisThrArgAlaLeuSerValArgGlnAla
1861  TTTATGCAACAGCCTTTCCTGGAACCCACGCACACACGTGCGCTCTCTGTGCGCCAAGCT

      ProLeuAlaAlaValGlyMetAspGlyLeuGluLysHisArgLeuValSerArgThrHis
1921  CCGCTGGCTGCGGTTGGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCAC

      SerSerProAlaAlaSerValLeuProHisProAlaMetAspArgProLeuGlnProGly
1981  TCTTCCCCTGCTGCCTCTGTTTTACCTCACCCAGCAATGGACCGCCCCCTCCAGCCTGGC

      SerAlaThrGlyIleAlaTyrAspProLeuMetLeuLysHisGlnCysValCysGlyAsn
2041  TCTGCAACTGGAATTGCCTATGACCCCTTGATGCTGAAACACCAGTGCGTTTGTGGCAAT

      SerThrThrHisProGluHisAlaGlyArgIleGlnSerIleTrpSerArgLeuGlnGlu
2101  TCCACCACCCACCCTGAGCATGCTGGACGAATACAGAGTATCTGGTCACGACTGCAAGAA

      ThrGlyLeuLeuAsnLysCysGluArgIleGlnGlyArgLysAlaSerLeuGluGluIle
2161  ACTGGGCTGCTAAATAAATGTGAGCGAATTCAAGGTCGAAAAGCCAGCCTGGAGGAAATA

      GlnLeuValHisSerGluHisHisSerLeuLeuTyrGlyThrAsnProLeuAspGlyGln
2221  CAGCTTGTTCATTCTGAACATCACTCACTGTTGTATGGCACCAACCCCCTGGACGGACAG

      LysLeuAspProArgIleLeuLeuGlyAspAspSerGlnLysPhePheSerSerLeuPro
2281  AAGCTGGACCCCAGGATACTCCTAGGTGATGACTCTCAAAAGTTTTTTTCCTCATTACCT
```

FIG. 22E

```
      CysGlyGlyLeuGlyValAspSerAspThrIleTrpAsnGluLeuHisSerSerGlyAla
2341  TGTGGTGGACTTGGGGTGGACAGTGACACCATTTGGAATGAGCTACACTCGTCCGGTGCT

      AlaArgMetAlaValGlyCysValIleGluLeuAlaSerLysValAlaSerGlyGluLeu
2401  GCACGCATGGCTGTTGGCTGTGTCATCGAGCTGGCTTCCAAAGTGGCCTCAGGAGAGCTG

      LysAsnGlyPheAlaValValArgProProGlyHisHisAlaGluGluSerThrAlaMet
2461  AAGAATGGGTTTGCTGTTGTGAGGCCCCCTGGCCATCACGCTGAAGAATCCACAGCCATG

      GlyPheCysPhePheAsnSerValAlaIleThrAlaLysTyrLeuArgAspGlnLeuAsn
2521  GGGTTCTGCTTTTTTAATTCAGTTGCAATTACCGCCAAATACTTGAGAGACCAACTAAAT

      IleSerLysIleLeuIleValAspLeuAspValHisHisGlyAsnGlyThrGlnGlnAla
2581  ATAAGCAAGATATTGATTGTAGATCTGGATGTTCACCATGGAAACGGTACCCAGCAGGCC

      PheTyrAlaAspProSerIleLeuTyrIleSerLeuHisArgTyrAspGluGlyAsnPhe
2641  TTTTATGCTGACCCCAGCATCCTGTACATTTCACTCCATCGCTATGATGAAGGGAACTTT

      PheProGlySerGlyAlaProAsnGluValArgPheIleSerLeuGluProHisPheTyr
2701  TTCCCTGGCAGTGGAGCCCCAAATGAGGTTCGGTTTATTTCTTTAGAGCCCCACTTTTAT

      LeuTyrLeuSerGlyAsnCysIleAla***
2761  TTGTATCTTTCAGGTAATTGCATTGCATGA
```

FIG. 22F

```
   1  GGGGAAGAGAGGCACAGACACAGATAGGAGAAGGGCACCGGCTGGAGCCACTTGCAGGAC
  61  TGAGGGTTTTTGCAACAAAACCCTAGCAGCCTGAAGAACTCTAAGCCAGATGGGGTGGCT

                                                MetHisSerMetIleSerSerValAspVal
 121  GGACGAGAGCAGCTCTTGGCTCAGCAAAGAATGCACAGTATGATCAGCTCAGTGGATGTG

      LysSerGluValProValGlyLeuGluProIleSerProLeuAspLeuArgThrAspLeu
 181  AAGTCAGAAGTTCCTGTGGGCCTGGAGCCCATCTCACCTTTAGACCTAAGGACAGACCTC

      ArgMetMetMetProValValAspProValValArgGluLysGlnLeuGlnGlnGluLeu
 241  AGGATGATGATGCCCGTGGTGGACCCTGTTGTCCGTGAGAAGCAATTGCAGCAGGAATTA

      LeuLeuIleGlnGlnGlnGlnGlnIleGlnLysGlnLeuLeuIleAlaGluPheGlnLys
 301  CTTCTTATCCAGCAGCAGCAACAAATCCAGAAGCAGCTTCTGATAGCAGAGTTTCAGAAA

      GlnHisGluAsnLeuThrArgGlnHisGlnAlaGlnLeuGlnGluHisIleLysGluLeu
 361  CAGCATGAGAACTTGACACGGCAGCACCAGGCTCAGCTTCAGGAGCATATCAAGGAACTT

      LeuAlaIleLysGlnGlnGlnGluLeuLeuGluLysGluGlnLysLeuGluGlnGlnArg
 421  CTAGCCATAAAACAGCAACAAGAACTCCTAGAAAAGGAGCAGAAACTGGAGCAGCAGAGG

      GlnGluGlnGluValGluArgHisArgArgGluGlnGlnLeuProProLeuArgGlyLys
 481  CAAGAACAGGAAGTAGAGAGGCATCGCAGAGAACAGCAGCTTCCTCCTCTCAGAGGCAAA

      AspArgGlyArgGluArgAlaValAlaSerThrGluValLysGlnLysLeuGlnGluPhe
 541  GATAGAGGACGAGAAAGGGCAGTGGCAAGTACAGAAGTAAAGCAGAAGCTTCAAGAGTTC

      LeuLeuSerLysSerAlaThrLysAspThrProThrAsnGlyLysAsnHisSerValSer
 601  CTACTGAGTAAATCAGCAACGAAAGACACTCCAACTAATGGAAAAAATCATTCCGTGAGC

      ArgHisProLysLeuTrpTyrThrAlaAlaHisHisThrSerLeuAspGlnSerSerPro
 661  CGCCATCCCAAGCTCTGGTACACGGCTGCCCACCACACATCATTGGATCAAAGCTCTCCA

      ProLeuSerGlyThrSerProSerTyrLysTyrThrLeuProGlyAlaGlnAspAlaLys
 721  CCCCTTAGTGGAACATCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAG

      AspAspPheProLeuArgLysThrAlaSerGluProAsnLeuLysValArgSerArgLeu
 781  GATGATTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCAGGTTA

      LysGlnLysValAlaGluArgArgSerSerProLeuLeuArgArgLysAspGlyAsnVal
 841  AAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGGAAGGATGGAAATGTT

      ValThrSerPheLysLysArgMetPheGluValThrGluSerSerValSerSerSerSer
 901  GTCACTTCATTCAAGAAGCGAATGTTTGAGGTGACAGAATCCTCAGTCAGTAGCAGTTCT

      ProGlySerGlyProSerSerProAsnAsnGlyProThrGlySerValThrGluAsnGlu
 961  CCAGGCTCTGGTCCCAGTTCACCAAACAATGGGCCAACTGGAAGTGTTACTGAAAATGAG

      ThrSerValLeuProProThrProHisAlaGluGlnMetValSerGlnGlnArgIleLeu
1021  ACTTCGGTTTTGCCCCCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTA

      IleHisGluAspSerMetAsnLeuLeuSerLeuTyrThrSerProSerLeuProAsnIle
1081  ATTCATGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCAACATT

      ThrLeuGlyLeuProAlaValProSerGlnLeuAsnAlaSerAsnSerLeuLysGluLys
1141  ACCTTGGGGCTTCCCGCAGTGCCATCCCAGCTCAATGCTTCGAATTCACTCAAAGAAAAG
```

FIG. 22G

```
      GlnLysCysGluThrGlnThrLeuArgGlnGlyValProLeuProGlyGlnTyrGlyGly
1201  CAGAAGTGTGAGACGCAGACGCTTAGGCAAGGTGTTCCTCTGCCTGGGCAGTATGGAGGC

      SerIleProAlaSerSerSerHisProHisValThrLeuGluGlyLysProProAsnSer
1261  AGCATCCCGGCATCTTCCAGCCACCCTCATGTTACTTTAGAGGGAAAGCCACCCAACAGC

      SerHisGlnAlaLeuLeuGlnHisLeuLeuLeuLysGluGlnMetArgGlnGlnLysLeu
1321  AGCCACCAGGCTCTCCTGCAGCATTTATTATTGAAAGAACAAATGCGACAGCAAAAGCTT

      LeuValAlaGlyGlyValProLeuHisProGlnSerProLeuAlaThrLysGluArgIle
1381  CTTGTAGCTGGTGGAGTTCCCTTACATCCTCAGTCTCCCTTGGCAACAAAAGAGAGAATT

      SerProGlyIleArgGlyThrHisLysLeuProArgHisArgProLeuAsnArgThrGln
1441  TCACCTGGCATTAGAGGTACCCACAAATTGCCCCGTCACAGACCCCTGAACCGAACCCAG

      SerAlaProLeuProGlnSerThrLeuAlaGlnLeuValIleGlnGlnGlnHisGlnGln
1501  TCTGCACCTTTGCCTCAGAGCACGTTGGCTCAGCTGGTCATTCAACAGCAACACCAGCAA

      PheLeuGluLysGlnLysGlnTyrGlnGlnGlnIleHisMetAsnLysLeuLeuSerLys
1561  TTCTTGGAGAAGCAGAAGCAATACCAGCAGCAGATCCACATGAACAAACTGCTTTCGAAA

      SerIleGluGlnLeuLysGlnProGlySerHisLeuGluGluAlaGluGluGluLeuGln
1621  TCTATTGAACAACTGAAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAG

      GlyAspGlnAlaMetGlnGluAspArgAlaProSerSerGlyAsnSerThrArgSerAsp
1681  GGGGACCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGAGCGAC

      SerSerAlaCysValAspAspThrLeuGlyGlnValGlyAlaValLysValLysGluGlu
1741  AGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTGAAGGTCAAGGAGGAA

      ProValAspSerAspGluAspAlaGlnIleGlnGluMetGluSerGlyGluGlnAlaAla
1801  CCAGTGGACAGTGATGAAGATGCTCAGATCCAGGAAATGGAATCTGGGGAGCAGGCTGCT

      PheMetGlnGlnValIleGlyLysAspLeuAlaProGlyPheValIleLysValIleIle
1861  TTTATGCAACAGGTAATAGGCAAAGATTTAGCTCCAGGATTTGTAATTAAAGTCATTATC

      ***
1921  TGAACATGAAATGCATTGCAGGTTTGGTAAATGGATATGATTTCCTATCAGTTTATATTT

1981  CTCTATGATTTGAGTTCAGTGTTTAAGGATTCTACCTAATGCAGATATATGTATATATCT
2041  ATATAGAGGTCTTTCTATATACTGATCTCTATATAGATATCAATGTTTCATTGAAAATCC
2101  ACTGGTAAGGAAATACCTGTTATACTAAAATTATGATACATAATATCTGAGCAGTTAATA
2161  GGCTTTAAATTTATCCCAAAGCCTGCTACACCAATTACTTCTAAAGAAAACAAATTCACT
2221  GTTATTTTGAGTTTATGTGTTGAGATCAGTGACTGCTGGATAGTCTCCCAGTCTGATCAA
2281  TGAAGCATTCGATTAGTTTTTGATTTTTTGCAACATCTAGAATTTAATTTTCACATCACT
2341  GTACATAATGTATCATACTATAGTCTTGAACACTGTTAAAGGTAGTCTGCCCCTTCCTTC
2401  CTCTCTCTTTTTTTTAGTTAAGTAGAAATGTTCTGGTCACCATGCCAGTAGTCCTAGGTTA
2461  TTGTGTAGGTTGCAATTGAACATATTAGGAATACAGGTGGTTTTAAATATATAGATGCAA
2521  ATTGCAGCACTACTTTAAATATTAGATTATGTCTCACATAGCACTGCTCATTTTACTTTT
2581  ATTTTGTGTAATTTGATGACACTGTCTATCAAAAAAGAGCAAATGAAGCAGATGCAAATG
2641  TTAGTGAGAAGTAATGTGCAGCATTATGGTCCAATCAGATACAATATTGTGTCTACAATT
2701  GCAAAAAACACAGTAACAGGATGAATATTATCTGATATCAAGTCAAAATCAGTTTGAAAA
2761  GAAGGTGTATCATATTTTATATTGTCACTAGAATCTCTTAAGTATAATTCCATAATGACA
2821  TGGGCATATACCGTAACATTCTGGCAAATAACAATTAGAAAAGATAGGTTTAACAAAAAA
2881  ATTTACTTGTATATAATGCACCTTCAGGAGGACTATGTCCTTTGATGCTATAAAATACAA
2941  ACAACTTTGAAGGCAACAGAAGACACTGTTTATTCAAGTCAGTTCTTTGTCAGGTTCCTG
3001  CTGTTCTCCTACAGAAAAGTGATTCTGTGAGGGTGAACAGGAAATGCCTTGTGGAAACAG
3061  GAAGTCCAAGTGATTCATGTACTGAGGAATGTAGGAAAAAAAATCTGAGGATAGTGCTTT
```

FIG. 22H

```
3121  ACTCTTTCTGTTTTTAAAGGGCACTCTATGAATTGATTTATTGTCTAAGAAAATAACACC
3181  ACAAGTAGGGAAATTGTTACGGAAGCTTTTCACTGGAACATTTCCTTCATATTCCCTTTT
3241  GATATGTTTACCTTGTTTTATAGGTTTACTTTTGTTAAGCTAGTTAAAGGTTCGTTGTAT
3301  TAAGACCCCTTTAATATGGATAATCCAAATTGACCTAGAATCTTTGTGAGGTTTTTTCTA
3361  TTAAAATATTTATATTTCTAAATCCGAGGTATTTCAAGGTGTAGTATCCTATTTCAAAGG
3421  AGATATAGCAGTTTTGCCAAATGTAGACATTGTTCAACTGTATGTTATTGGCACGTGTTG
3481  TTTACATTTTGCTGTGACATTTAAAAATATTTCTTTAAAAATGTTACTGCTAAAGATACA
3541  TTATCCTTTTTTTAAAAAGTCTCCATTCAAATTAAATTAACATAACTAGAAGTTAGAAAGT
3601  TTAAAAGTTTTCCACATAATGAAAGTCCTTCTGATAATTTGACAAATAGCTATAATAGGA
3661  ACACTCCCTATCACCAACATATTTTGGTTAGTATATTCCTTCATATTAAAATGACTTTTT
3721  GTCAGTTGTTTTTGCATTAAAAATATGGCATGCCTAAGATAAAATTGTATATTTTTTCCAT
3781  CTCATAAATATTCATTTTCTTCAAAGTCTTTTTTCAATCTCATAAAAAAGGGATAGTGCA
3841  TCTTTTAAAATACATTTTATTTGGGGAGGAACATGTGGCTGAGCAGACTTTTGTATAATA
3901  TTACTTCAAAGATATGTAATCACAAACAAAAAAAACTATTTTTTATAATGTCATTTGAGA
3961  GAGTTTCATCAGTACAGTTGGTGGACGTTAATTGTTTGAATTTGATAGTCTTTGAATTTA
4021  ATCAAGAAACTACCTGGAACCAGTGAAAAGGAAAGCTGGACTTAAATAATCTTAGAATTA
4081  ATTGATAAATGTCTCTTTTAAAATCTACTGTATTTATTATAATTTACACCCTTGAAGGTG
4141  ATCTCTTGTTTTGTGTTGTAAATATATTGTTTGTATGTTTCCCTTCTTGCCTTCTGTTAT
4201  AAGTCTCTTCCTTTCTCAAATAAAGTTTTTTTTAAAAG
```

FIG. 22I

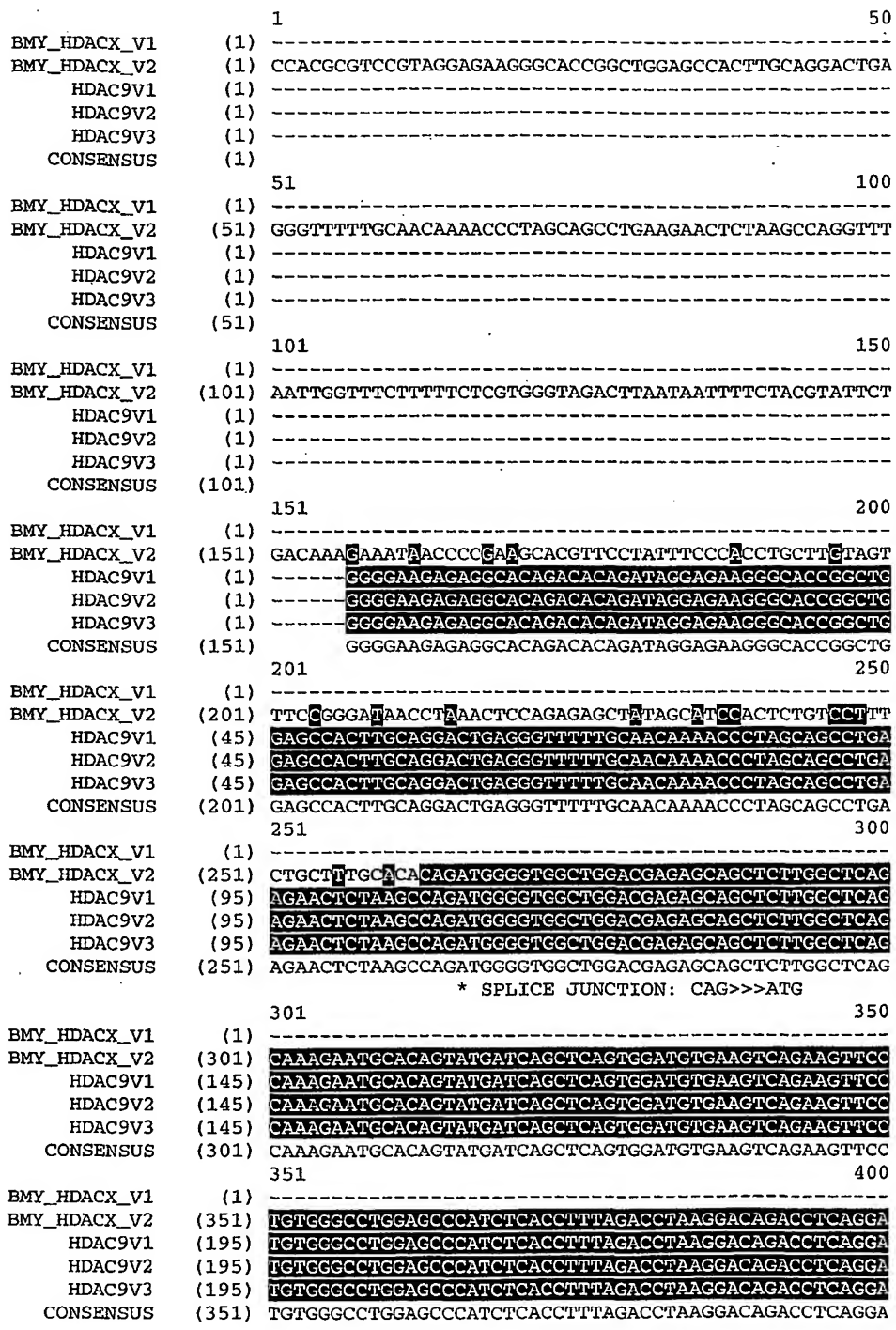



FIG. 23A

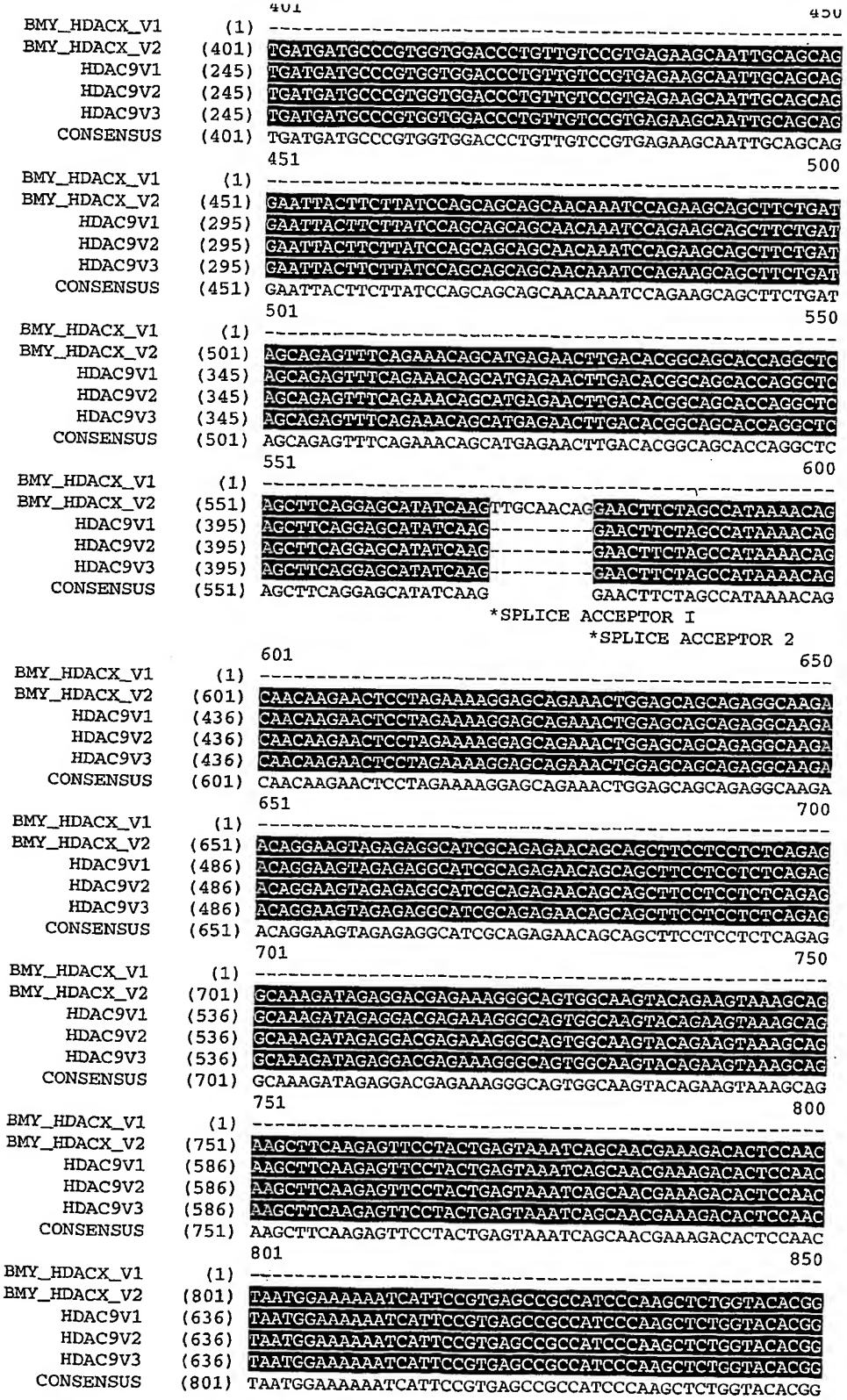



FIG. 23B

```
                        851                                                 900
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2    (851)   CTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA
     HDAC9V1    (686)   CTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA
     HDAC9V2    (686)   CTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA
     HDAC9V3    (686)   CTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA
   CONSENSUS    (851)   CTGCCCACCACACATCATTGGATCAAAGCTCTCCACCCCTTAGTGGAACA
                        901                                                 950
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2    (901)   TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGA
     HDAC9V1    (736)   TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGA
     HDAC9V2    (736)   TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGA
     HDAC9V3    (736)   TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGA
   CONSENSUS    (901)   TCTCCATCCTACAAGTACACATTACCAGGAGCACAAGATGCAAAGGATGA
                        951                                                1000
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2    (951)   TTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCA
     HDAC9V1    (786)   TTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCA
     HDAC9V2    (786)   TTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCA
     HDAC9V3    (786)   TTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCA
   CONSENSUS    (951)   TTTCCCCCTTCGAAAAACTGCCTCTGAGCCCAACTTGAAGGTGCGGTCCA
                        1001                                               1050
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2   (1001)   GGTTAAAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGG
     HDAC9V1    (836)   GGTTAAAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGG
     HDAC9V2    (836)   GGTTAAAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGG
     HDAC9V3    (836)   GGTTAAAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGG
   CONSENSUS   (1001)   GGTTAAAACAGAAAGTGGCAGAGAGGAGAAGCAGCCCCTTACTCAGGCGG
                        1051                                               1100
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2   (1051)   AAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGAATGTTTGAGGTGAC
     HDAC9V1    (886)   AAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGAATGTTTGAGGTGAC
     HDAC9V2    (886)   AAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGAATGTTTGAGGTGAC
     HDAC9V3    (886)   AAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGAATGTTTGAGGTGAC
   CONSENSUS   (1051)   AAGGATGGAAATGTTGTCACTTCATTCAAGAAGCGAATGTTTGAGGTGAC
                        1101                                               1150
BMY_HDACX_V1      (1)   ----------------------------------------------------
BMY_HDACX_V2   (1101)   AGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCACCAA
     HDAC9V1    (936)   AGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCACCAA
     HDAC9V2    (936)   AGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCACCAA
     HDAC9V3    (936)   AGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCACCAA
   CONSENSUS   (1101)   AGAATCCTCAGTCAGTAGCAGTTCTCCAGGCTCTGGTCCCAGTTCACCAA
                        1151                                               1200
BMY_HDACX_V1      (1)   ----------------------GCTGAAAATGAGACTTCGGTTTTGCCC
BMY_HDACX_V2   (1151)   ACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC
     HDAC9V1    (986)   ACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC
     HDAC9V2    (986)   ACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC
     HDAC9V3    (986)   ACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC
   CONSENSUS   (1151)   ACAATGGGCCAACTGGAAGTGTTACTGAAAATGAGACTTCGGTTTTGCCC
                        1201                                               1250
BMY_HDACX_V1     (28)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
BMY_HDACX_V2   (1201)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
     HDAC9V1   (1036)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
     HDAC9V2   (1036)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
     HDAC9V3   (1036)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
   CONSENSUS   (1201)   CCTACCCCTCATGCCGAGCAAATGGTTTCACAGCAACGCATTCTAATTCA
                        1251                                               1300
BMY_HDACX_V1     (78)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
BMY_HDACX_V2   (1251)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
     HDAC9V1   (1086)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
     HDAC9V2   (1086)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
     HDAC9V3   (1086)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
   CONSENSUS   (1251)   TGAAGATTCCATGAACCTGCTAAGTCTTTATACCTCTCCTTCTTTGCCCA
```

FIG. 23C

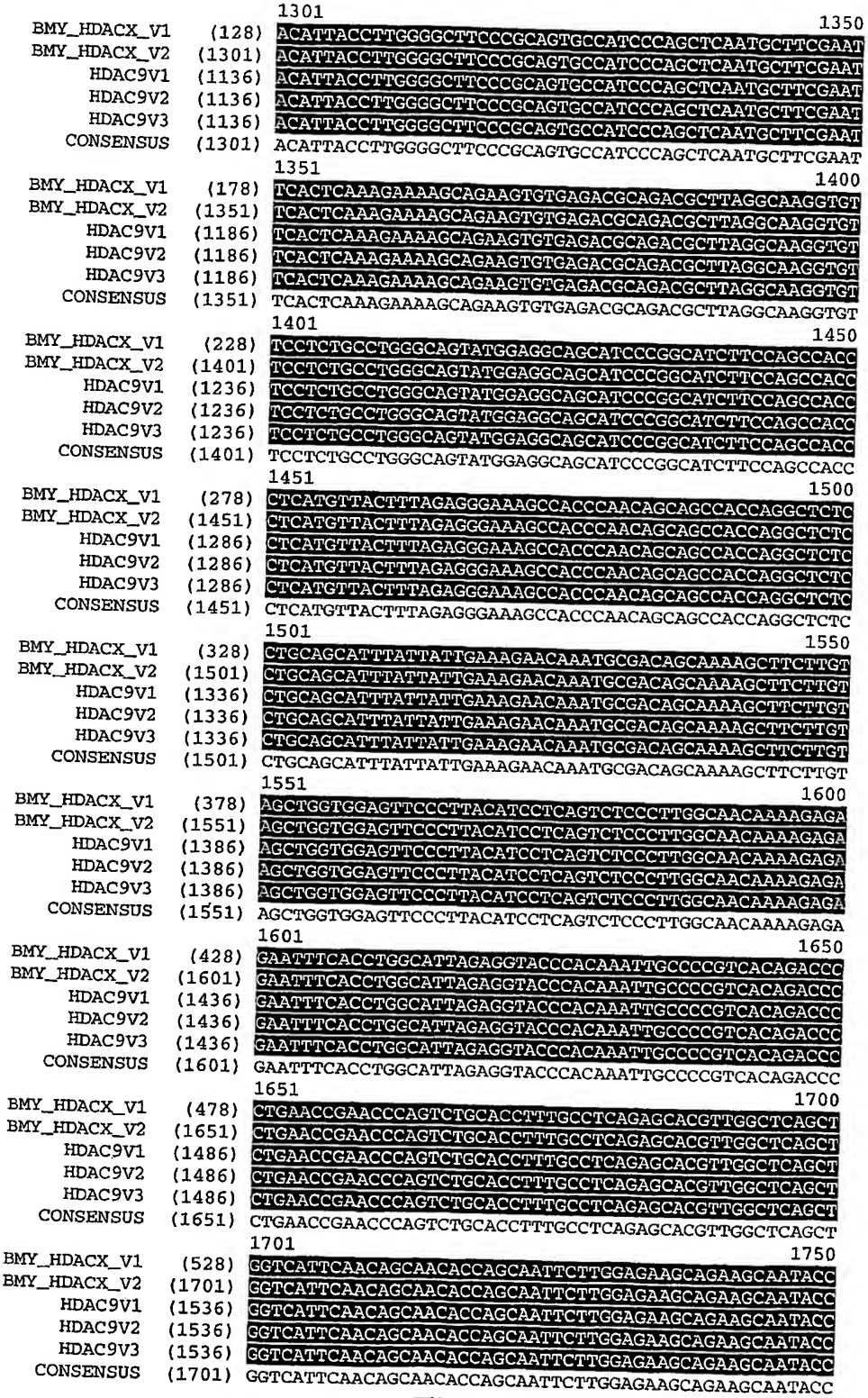



FIG. 23D

```
                        1751                                                1800
BMY_HDACX_V1    (578)   AGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTG
BMY_HDACX_V2    (1751)  AGCAGCAGATCCACATGAACAAAGAATTGCCTATGACCCCTTGATGCTGA
     HDAC9V1    (1586)  AGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTG
     HDAC9V2    (1586)  AGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTG
     HDAC9V3    (1586)  AGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTG
   CONSENSUS    (1751)  AGCAGCAGATCCACATGAACAAACTGCTTTCGAAATCTATTGAACAACTG
                                              *SPLICE JUNCTION:
                                               CAAA>>GAAA OR CTGC
                        1801                                               .1850
BMY_HDACX_V1    (628)   AAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGA
BMY_HDACX_V2    (1801)  AACACCAGTGCGTTTGTGGCAATTCCACCACCCACCCTGAGCATGCTGGA
     HDAC9V1    (1636)  AAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGA
     HDAC9V2    (1636)  AAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGA
     HDAC9V3    (1636)  AAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGA
   CONSENSUS    (1801)  AAGCAACCAGGCAGTCACCTTGAGGAAGCAGAGGAAGAGCTTCAGGGGGA
                        1851                                                1900
BMY_HDACX_V1    (678)   CCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGA
BMY_HDACX_V2    (1851)  CGAATACAGAGTATCTGGTCACGACTGCAAGAAACTGGGCTGCTAAATAA
     HDAC9V1    (1686)  CCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGA
     HDAC9V2    (1686)  CCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGA
     HDAC9V3    (1686)  CCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGA
   CONSENSUS    (1851)  CCAGGCGATGCAGGAAGACAGAGCGCCCTCTAGTGGCAACAGCACTAGGA
                        1901                                                1950
BMY_HDACX_V1    (728)   GCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTG
BMY_HDACX_V2    (1901)  ATGTGAGCGAATTCAAGGTCGAAAAGCCAGCCTGGAGGAAATACAGCTTG
     HDAC9V1    (1736)  GCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTG
     HDAC9V2    (1736)  GCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTG
     HDAC9V3    (1736)  GCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTG
   CONSENSUS    (1901)  GCGACAGCAGTGCTTGTGTGGATGACACACTGGGACAAGTTGGGGCTGTG
                        1951                                                2000
BMY_HDACX_V1    (778)   AAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGA
BMY_HDACX_V2    (1951)  TTCATTCTGAACATCACGTCACTGTTGTATGCCACCAACCCCCTGGACGGA
     HDAC9V1    (1786)  AAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGA
     HDAC9V2    (1786)  AAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGA
     HDAC9V3    (1786)  AAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGA
   CONSENSUS    (1951)  AAGGTCAAGGAGGAACCAGTGGACAGTGATGAAGATGCTCAGATCCAGGA
                        2001                                                2050
BMY_HDACX_V1    (828)   AATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGCCTTTCCTGGAAC
BMY_HDACX_V2    (2001)  CAGAAGCTGGACCCCAGGATACTCCTAGGTGATGACTCTCAAAAGTTTTT
     HDAC9V1    (1836)  AATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGCCTTTCCTGGAAC
     HDAC9V2    (1836)  AATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGCCTTTCCTGGAAC
     HDAC9V3    (1836)  AATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGGTAATAGGCAAAG
   CONSENSUS    (2001)  AATGGAATCTGGGGAGCAGGCTGCTTTTATGCAACAGCCTTTCCTGGAAC
                                                        *SPLICE JUNCTION:
                                                        CAG>>>CCT OR GTA
                        2051                                                2100
BMY_HDACX_V1    (878)   CCACGCACACACGTGCGCTCTCTGTGCGCCAAGCTCCGCTGGCTGCGGTT
BMY_HDACX_V2    (2051)  TTCCTCATTACCTTGTGGTGGACTTGGGGTGGACAGTGACACCATTTGGA
     HDAC9V1    (1886)  CCACGCACACACGTGCGCTCTCTGTGCGCCAAGCTCCGCTGGCTGCGGTT
     HDAC9V2    (1886)  CCACGCACACACGTGCGCTCTCTGTGCGCCAAGCTCCGCTGGCTGCGGTT
     HDAC9V3    (1886)  ATTTAGCTCCAGCATTTGTAATTAAAGTCATTATCTGAACATGAAATGCA
   CONSENSUS    (2051)  CCACGCACACACGTGCGCTCTCTGTGCGCCAAGCTCCGCTGGCTGCGGTT
                        2101                                                2150
BMY_HDACX_V1    (928)   GGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCACTCTTC
BMY_HDACX_V2    (2101)  ATGAGCTACACTCGTCCGGTGCTGCACGCATGGCTGTTGGCTGTGTCATC
     HDAC9V1    (1936)  GGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCACTCTTC
     HDAC9V2    (1936)  GGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCACTCTTC
     HDAC9V3    (1936)  TTGCAGGTTTGGTAAATGGATATGATTTCCTATCAGTTTATATTTCTCTA
   CONSENSUS    (2101)  GGCATGGATGGATTAGAGAAACACCGTCTCGTCTCCAGGACTCACTCTTC
```

FIG. 23E

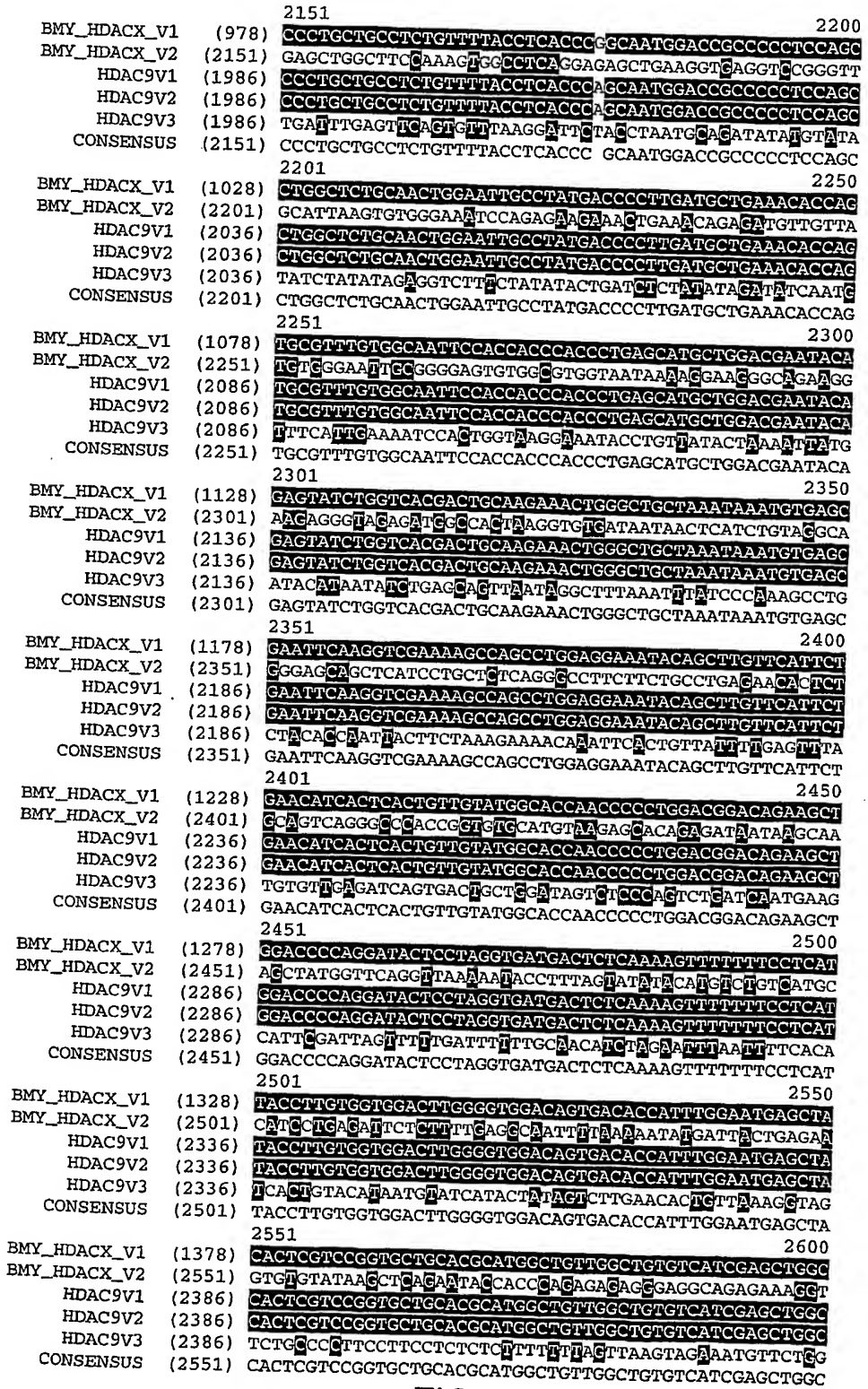



FIG. 23F

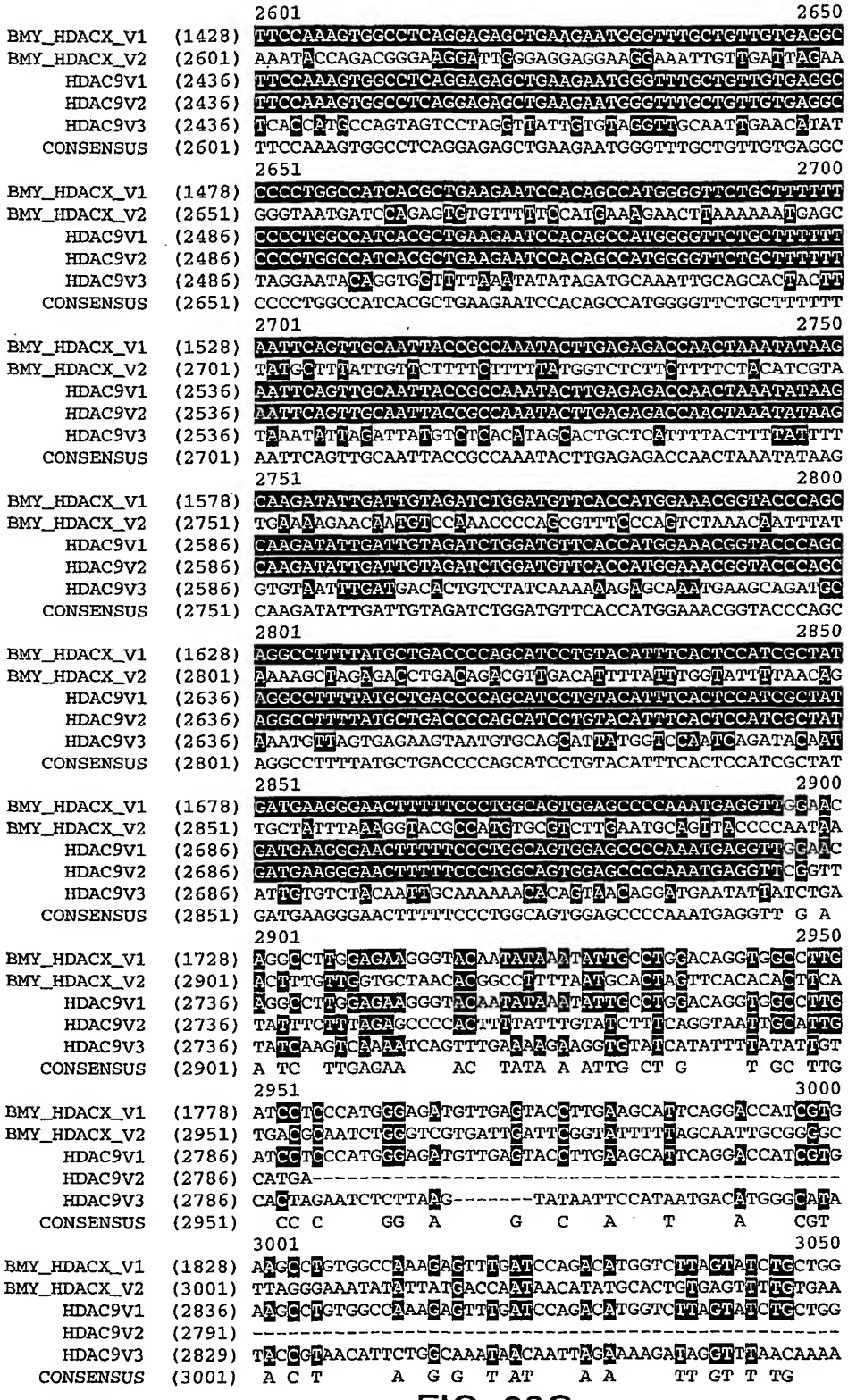



FIG. 23G

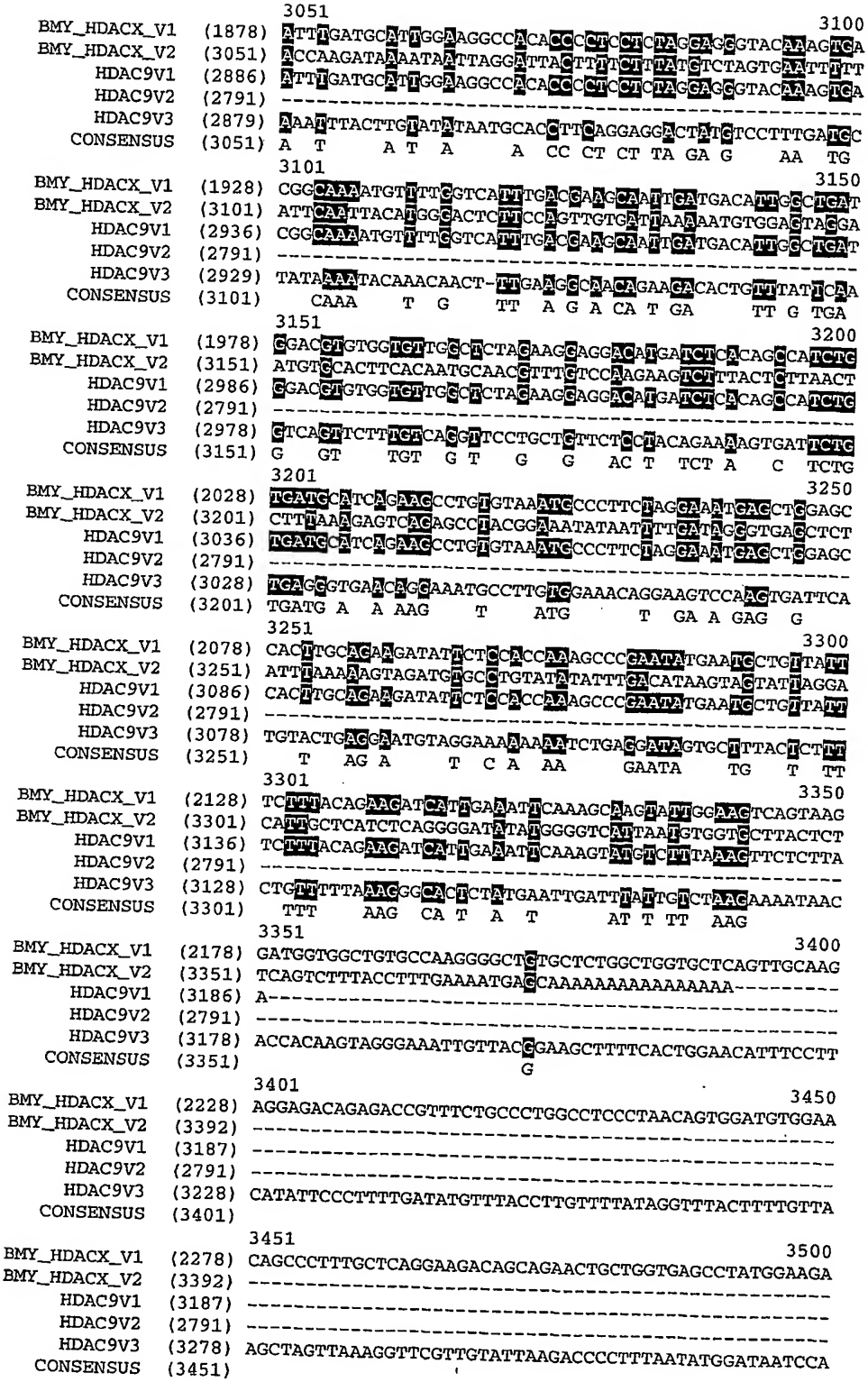



FIG. 23H

```
                        3501                                                3550
BMY_HDACX_V1   (2328) GGAGCCAGCCTTGTGAAGTGCCAAGTCCCCCTCTGATATTTCCTGTGTGT
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3328) AATTGACCTAGAATCTTTGTGAGGTTTTTTCTATTAAAATATTTATATTT
    CONSENSUS  (3501)
                        3551                                                3600
BMY_HDACX_V1   (2378) GACATCATTGTGTATCCCCCCACCCCAGTACCCTCAGACATGTCTTGTCT
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3378) CTAAATCCGAGGTATTTCAAGGTGTAGTATCCTATTTCAAAGGAGATATA
    CONSENSUS  (3551)
                        3601                                                3650
BMY_HDACX_V1   (2428) GCTGCCTGGGTGGCACAGATTCAATGGAACATAAACACTGGGCACAAAAT
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3428) GCAGTTTTGCCAAATGTAGACATTGTTCAACTGTATGTTATTGGCACGTG
    CONSENSUS  (3601)
                        3651                                                3700
BMY_HDACX_V1   (2478) TCTGAACAGCAGCTTCACTTGTTCTTTGGATGGACTTGAAAGGGCATTAA
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3478) TTGTTTACATTTTGCTGTGACATTTAAAAATATTTCTTTAAAAATGTTAC
    CONSENSUS  (3651)
                        3701                                                3750
BMY_HDACX_V1   (2528) AGATTCCTTAAACGTAACCGCTGTGATTCTAGAGTTACAGTAAACCACGA
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3528) TGCTAAAGATACATTATCCTTTTTTAAAAAGTCTCCATTCAAATTAAATT
    CONSENSUS  (3701)
                        3751                                                3800
BMY_HDACX_V1   (2578) TTGGAAGAAACTGCTTCCAGCATGCTTTTAATATGCTGGGTGACCCACTC
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3578) AACATAACTAGAAGTTAGAAAGTTTAAAAGTTTTCCACATAATGAAAGTC
    CONSENSUS  (3751)
                        3801                                                3850
BMY_HDACX_V1   (2628) CTAGACACCAAGTTTGAACTAGAAACATTCAGTACAGCACTAGATATTGT
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3628) CTTCTGATAATTTGACAAATAGCTATAATAGGAACACTCCCTATCACCAA
    CONSENSUS  (3801)
                        3851                                                3900
BMY_HDACX_V1   (2678) TAATTTCAGAAGCTATGACAGCCAGTGAAATTTTGGGCAAAACCTGAGAC
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3678) CATATTTTGGTTAGTATATTCCTTCATATTAAAATGACTTTTTGTCAGTT
    CONSENSUS  (3851)
                        3901                                                3950
BMY_HDACX_V1   (2728) ATAGTCATTCCTGACATTCTGATCAGCTTTTTTTGGGGTAATTTGTTTTT
BMY_HDACX_V2   (3392) ----------------------------------------------------
      HDAC9V1  (3187) ----------------------------------------------------
      HDAC9V2  (2791) ----------------------------------------------------
      HDAC9V3  (3728) GTTTTGCATTAAAAATATGGCATGCCTAAGATAAAATTGTATATTTTTTC
    CONSENSUS  (3901)
```

FIG. 23I

```
                        3951                                                4000
BMY_HDACX_V1   (2778) CAAACAGTCTTAACTTGTTTACAAGATTTGCTTTTAGCTATGAACGGATC
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (3778) CATCTCATAAATATTCATTTTCTTCAAAGTCTTTTTTCAATCTCATAAAA
    CONSENSUS  (3951)
                        4001                                                4050
BMY_HDACX_V1   (2828) GTAATTCCACCCAGAATGTAATGTTTCTTGTTTGTTTGTTTTGTTTTGTT
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (3828) AAGGGATAGTGCATCTTTTAAAATACATTTTATTTGGGGAGGAACATGTG
    CONSENSUS  (4001)
                        4051                                                4100
BMY_HDACX_V1   (2878) AGGGTTTTTTTCTCAACTTTAACACACAGTTCAACTGTTCCTAGTAAAAG
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (3878) GCTGAGCAGACTTTTGTATAATATTACTTCAAAGATATGTAATCACAAAC
    CONSENSUS  (4051)
                        4101                                                4150
BMY_HDACX_V1   (2928) TTCAAGATGGAGGAACTAGCATGAGGCTTTTTTCAGTATCTCGAAGTCCA
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (3928) AAAAAAAACTATTTTTTATAATGTCATTTGAGAGAGTTTCATCAGTACAG
    CONSENSUS  (4101)
                        4151                                                4200
BMY_HDACX_V1   (2978) AATGCCAAAGGAACCTCACACACTGTTTGTAATGGTGCAATATTTTATAT
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (3978) TTGGTGGACGTTAATTGTTTGAATTTGATAGTCTTTGAATTTAATCAAGA
    CONSENSUS  (4151)
                        4201                                                4250
BMY_HDACX_V1   (3028) CACTTTTTTTTAAACATCCCCAACATCTTTGTGTTCTCACACACAGGCAA
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (4028) AACTACCTGGAACCAGTGAAAAGGAAAGCTGGACTTAAATAATCTTAGAA
    CONSENSUS  (4201)
                        4251                                                4300
BMY_HDACX_V1   (3078) TTTGCAATGTTGCAATTGTGTTGGAGAATGAAGTCCCCCCACCTCCCAGC
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (4078) TTAATTGATAAATGTCTCTTTTAAAATCTACTGTATTTATTATAATTTAC
    CONSENSUS  (4251)
                        4301                                                4350
BMY_HDACX_V1   (3128) CACACACACATCCTTTGTTCTCATGACAGTAGGTCTGAGCAAATGTTCCA
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (4128) ACCCTTGAAGGTGATCTCTTGTTTTGTGTTGTAAATATATTGTTTGTATG
    CONSENSUS  (4301)
                        4351                                                4400
BMY_HDACX_V1   (3178) CCAAGCATTTTCAGTGTCTTTGAAAAGCACGTAACTTTTCAAAGGTGGTC
BMY_HDACX_V2   (3392) ------------------------------------------------------
      HDAC9V1  (3187) ------------------------------------------------------
      HDAC9V2  (2791) ------------------------------------------------------
      HDAC9V3  (4178) TTTCCCTTCTTGCCTTCTGTTATAAGTCTCTTCCTTTCTCAAATAAAGTT
    CONSENSUS  (4351)
```

FIG. 23J

```
                          4401                                                4450
BMY_HDACX_V1   (3228) TTAATTTGCTGCATATCTATCAAGGACTTATTCACTCACCTTTCCTTTTC
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4228) TTTTTTAAAAG-------------------------------------
   CONSENSUS   (4401)
                          4451                                                4500
BMY_HDACX_V1   (3278) TGCCCTCTATCAATTGATTTCTTCTTACCTTTCATCATTCATTCCTTCCT
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4451)
                          4501                                                4550
BMY_HDACX_V1   (3328) TTAGAAAAACTGAAGATTACCCATAATCTCCTCTTATTACTTGAGGGCCT
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4501)
                          4551                                                4600
BMY_HDACX_V1   (3378) TGACTATTTAGTTTATTTTGTTTACTTTACAGGTTAACACAGTTGTTTTG
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4551)
                          4601                                                4650
BMY_HDACX_V1   (3428) TCTGATTGCATTTTATTAACTGTGAAGCCGTTGAAATGAATATCACTTAA
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4601)
                          4651                                                4700
BMY_HDACX_V1   (3478) GCAACGTTGCTAAATTTCTATGTGTTTGAAATGTGTTAATGAAGGCACTG
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4651)
                          4701                                                4750
BMY_HDACX_V1   (3528) CTTATTTGTAGTCACCTTGAACTGACTTAACCTAGAAGCTGTGCCTTCTT
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4701)
                          4751                                                4800
BMY_HDACX_V1   (3578) GTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
BMY_HDACX_V2   (3392) ------------------------------------------------
     HDAC9V1   (3187) ------------------------------------------------
     HDAC9V2   (2791) ------------------------------------------------
     HDAC9V3   (4239) ------------------------------------------------
   CONSENSUS   (4751)
                          4801                4823
BMY_HDACX_V1   (3628) AAAAAAAAAAAAAAAAAAAAAAA
BMY_HDACX_V2   (3392) -----------------------
     HDAC9V1   (3187) -----------------------
     HDAC9V2   (2791) -----------------------
     HDAC9V3   (4239) -----------------------
   CONSENSUS   (4801)
```

FIG. 23K

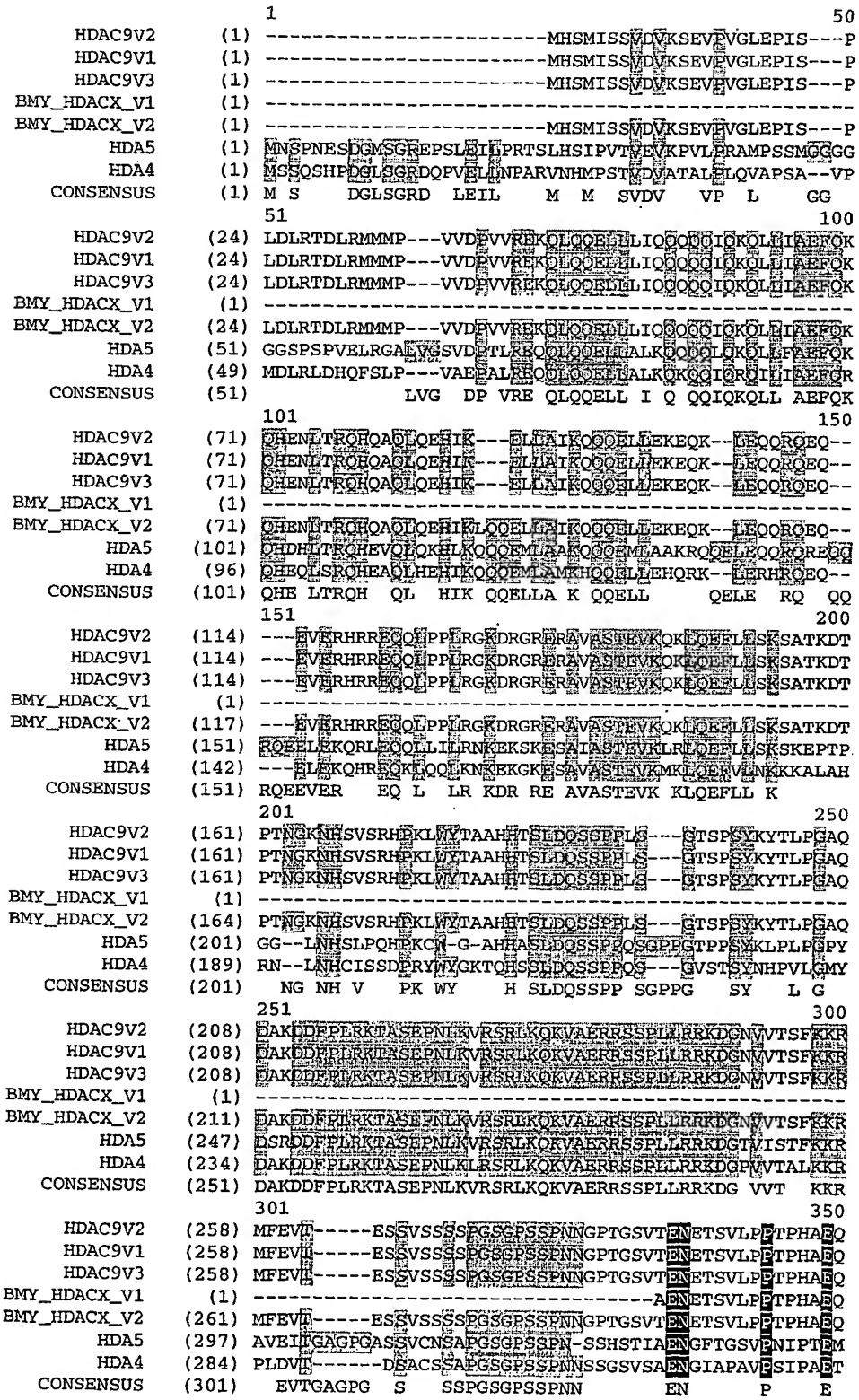



FIG. 24A

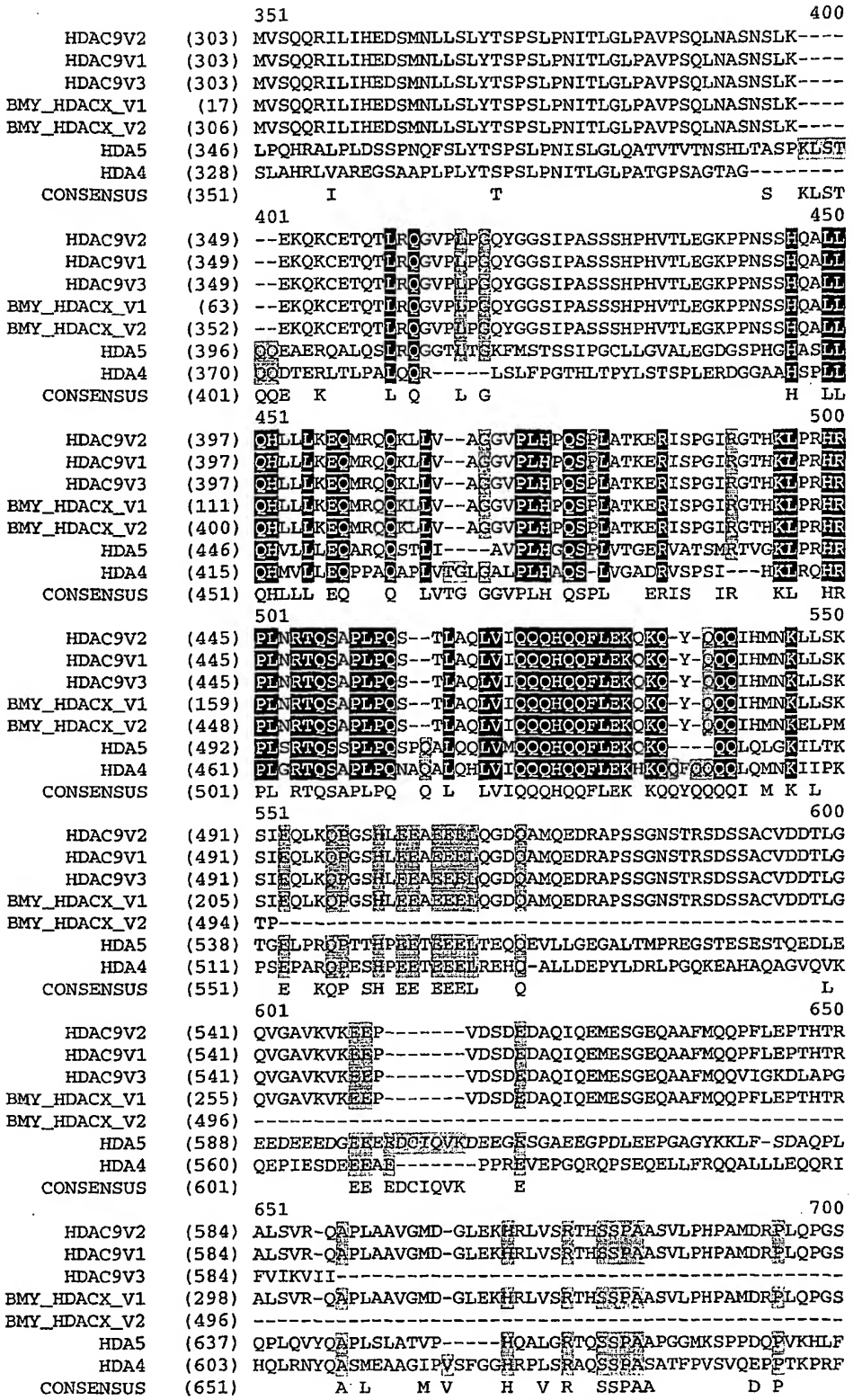



FIG. 24B

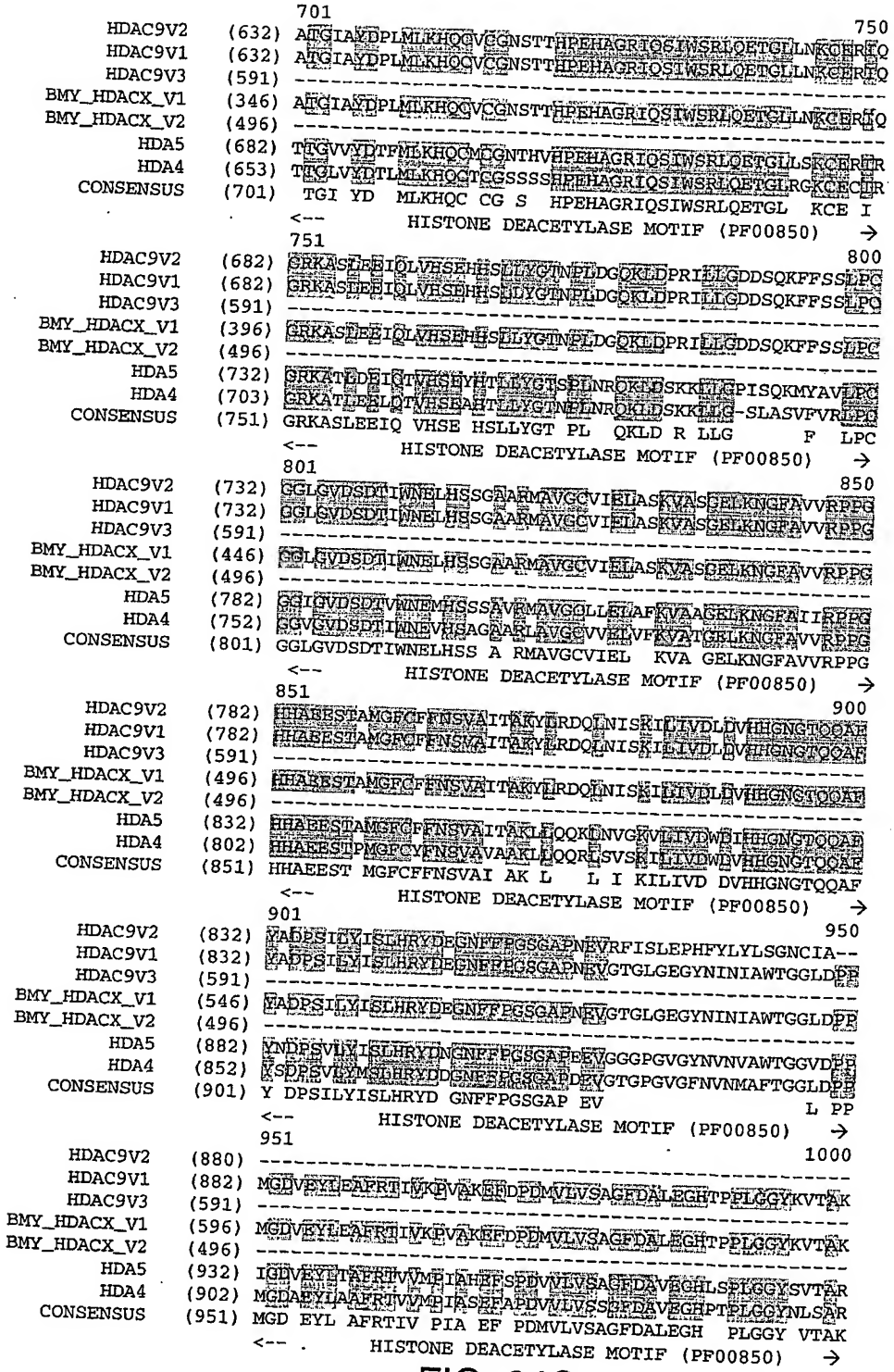



FIG. 24C

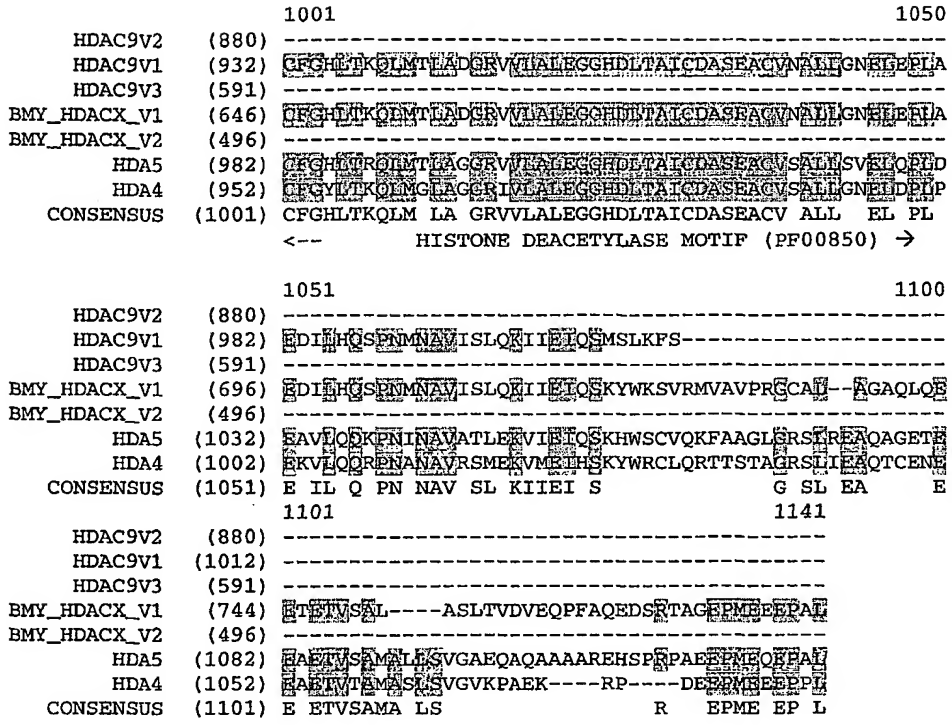



FIG. 24D

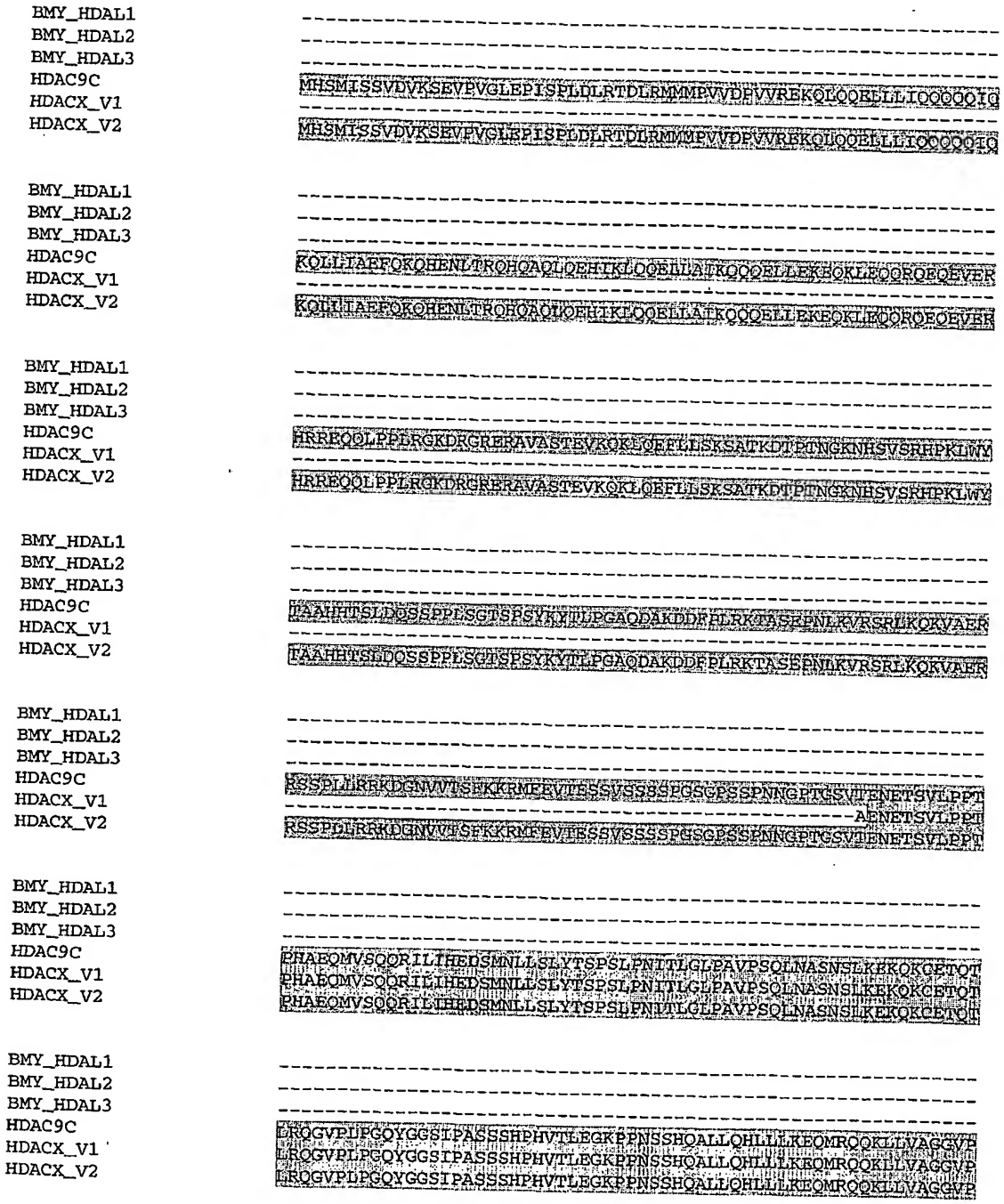



FIG. 25A

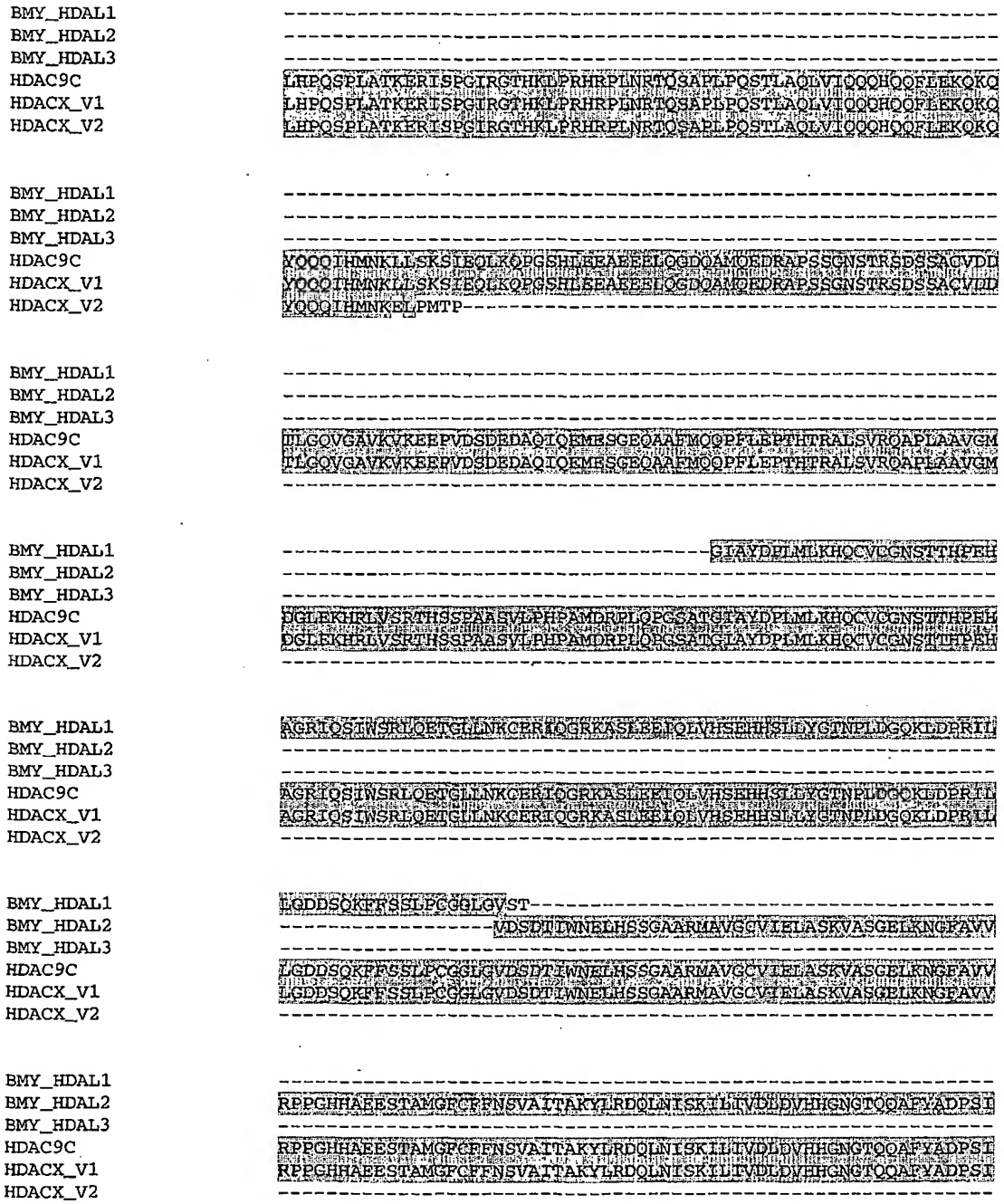



FIG. 25B

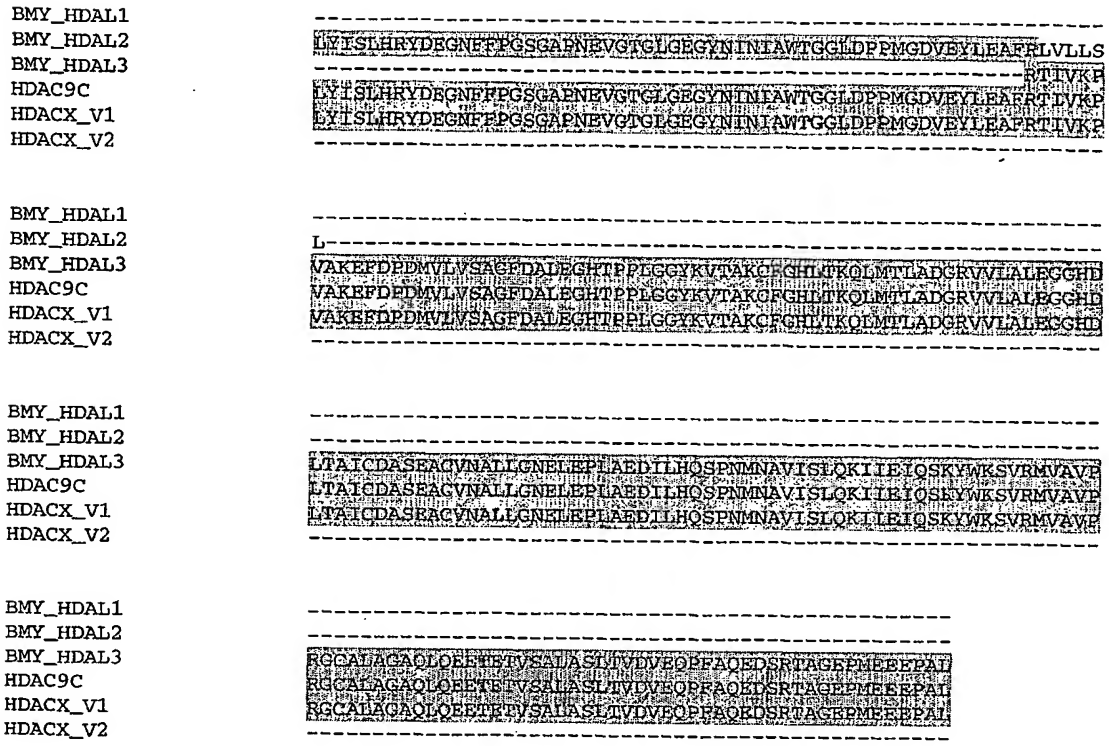



FIG. 25C

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(74) Agents: **D'AMICO, Stephen** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Route 206 and Provinceline Road, Princeton, NJ 08543-4000 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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WO 2002/102323 A3

(54) Title: NOVEL HUMAN HISTONE DEACETYLASES

(57) Abstract: The present invention relates to newly discovered human histone deacetylases (HDACs), also referred to as histone deacetylase-like polypeptides. The polynucleotide sequences and encoded polypeptides of the novel HDACs are encompassed by the invention, as well as vectors comprising these polynucleotides and host cells comprising these vectors. The invention also relates to antibodies that bind to the disclosed HDAC polypeptides, and methods employing these antibodies. Also related are methods of screening for modulators, such as inhibitors or antagonists, or agonists. The invention also relates to diagnostic and therapeutic applications which employ the disclosed HDAC polynucleotides, polypeptides, and antibodies, and HDAC modulators. Such applications can be used with diseases and disorders associated with abnormal cell growth or proliferation, cell differentiation, and cell survival, e.g., neoplastic cell growth, and especially breast and prostate cancers or tumors.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/19560

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/11, 15/85, 15/86, 1/20, 9/00, 15/63; C07H 21/04; C12Q 1/68; G01N 33/543, 577
US CL : 536/23.1, 24.5, 24.33; 435/325, 252.1, 193, 320.1, 69.1, 6, 7.1, 7.23; 436/501, 518

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.1, 24.5, 24.33; 435/325, 252.1, 193, 320.1, 69.1, 6, 7.1, 7.23; 436/501, 518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WANG et al., HDAC4, a human histone deacetylase related to yeast HDA1, is a transcritional corepressor, Molecular and Cellular Biology, November 1999, vol. 19, pages 7816-7827 | 1-20 |
| A | ZHOU et al., Cloning and characterization of a histone deacetylase, HDAC9, Proc. Natl. Acad. Sci. USA, 11 September 2001, vol. 98, pages 10572-10577. | 1-20 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
18 January 2005 (18.01.2005)

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MISOOK YU, Ph.D.

Telephone No. 571-272-1600

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PCT/US02/19560

# INTERNATIONAL SEARCH REPORT

**Continuation of B. FIELDS SEARCHED Item 3:**
Dialog(5, 155), West (USPT, DWPI), sequence databases
Search terms: histone deacetylases, cancer diagnosis, SEQ ID NOs 2, 95, 87, 96, 4, 5, 83.